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Congenital Aniridia*

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INTRODUCTION

CONGENITAL ANIRIDIA is a condition characterized by bilateral absence of the iris. It is accompanied by ocular nystagmus and deficient macular vision, and frequently complicated by cataracts and glaucoma, leading to very marked impairment of sight at an early age. The condition has been reported by many investigators to follow the pattern of autosomal dominant inheritance (reviews in Bell, 1932, Møllenbach, 1947, and Sorsby, 1951). Since the phenotype is recognizable at birth and presents no diagnostic difficulties, it lends itself well to studies concerned with such aspects of human genetics as the estimation of mutation rates and selection coefficients.

The present study was undertaken as one of a series designed to determine the impact and population dynamics of selected inherited diseases in the state of Michigan. In addition to providing data concerning the frequency and relative fertility of the aniridia phenotype and an estimate of the rate with which this phenotype appears as a result of mutation, we shall present evidence that in families in which the aniridia gene is segregating, there is a significant departure from the expected 1:1 ratio.

THE COLLECTION OF MATERIAL

In order to determine the frequency of aniridia, a roster of affected individuals residing in the lower peninsula of the state of Michigan was compiled. (Hereinafter, whenever "Michigan" is used it is understood to refer to the lower peninsula only.) The aniridia cases in the present study were ascertained from the following sources, given in approximate chronological order as the study progressed:

1. Referrals to the Department of Human Genetics from the Department of Ophthalmology at the University of Michigan Medical Center, from June, 1942 to January, 1960.
2. Survey of University of Michigan Hospital records with a diagnosis of congenital aniridia for patients seen between July, 1928 and March, 1956.
3. Survey of approximately 3,600 diagnostic cards at the Michigan School for the Blind, Lansing, of all children enrolled between 1925 and 1956.

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4. Survey of approximately 3,000 current and old records filed in the State Department of Social Welfare, Division of Services for the Blind, Lansing.
5. Survey of approximately 7,000 current and old records filed in the State Department of Social Welfare, Aid to the Blind, Lansing. (Although a few names were duplicated in the Aid to the Blind and Division of Services for the Blind files, they were treated as separate ascertainments since the amount of overlapping between these two sources was small.)
6. Survey of 1,805 current and old files in the Sight Saving and Braille classes of the Department of Special Education, Detroit Public Schools.
7. Follow-up of 11 records of children reported to have congenital defects of the iris and listed with the Vision Section, Division of Maternal and Child Health, Michigan Department of Health, Lansing.
8. Survey of 209 cases of congenital eye defects and diseases of the orbit listed in the files of the Office of Vocational Rehabilitation, State Department of Public Instruction, Lansing.
9. Replies from inquiries to Jackson, Dearborn, Kalamazoo, and Grand Rapids Boards of Education, Sight Saving and Braille classes, regarding possible aniridic children listed in their diagnostic files.
10. Correspondence with the National Society for the Prevention of Blindness, New York City. (The reply stated that the only Michigan cases in the Society records were referred through the State School for the Blind and the special sight saving classes which we had already contacted.)
11. Letters to records librarians of 165 hospitals in Michigan, requesting names of individuals listed as in-patients or out-patients, with a diagnosis of congenital aniridia. (One hundred and fifty librarians replied to our letter, a response rate of 0.91.)
12. Correspondence with 122 board-certified ophthalmologists in Michigan, requesting names of aniridia cases seen in their private practices, of whom 61 (50 per cent) replied to our query. (We did not send letters to 41 ophthalmologists to whom we had previously written concerning patients in our study, thus decreasing our chances for reascertainment.)
13. Information volunteered by two individuals who were aware of our study but were not contacted by us.

Table 1 summarizes the number of cases obtained from each of the sources listed above. Through these various channels, 109 aniridics became known to us through 171 "separate" ascertainments. Subsequent family studies led to information on 67 additional affected individuals, giving a total number of 176 cases, the material on which this study is based. The sex ratio of the entire group was 82 males to 94 females ($\chi^2 = 0.82$; $.40 > P > .30$).

The treatment of certain types of population data is greatly simplified if, where some affected individuals are repeatedly ascertained, each ascertainment is independent of the other. Unfortunately, in studies such as the present, where the need is to build up as complete a roster as possible, repeated ascertainments are seldom entirely independent of one another. We have used the

TABLE 1. NUMBER OF PROBANDS AND REASCERTAINMENTS OBTAINED FROM VARIOUS SOURCES

Source of ascertainment	Probands	Reascer-tainments	Total ascer-tainments
1. Ophthalmology Department, University Hospital	20	2	22
2. University Hospital Records Survey	15	12	27
3. Michigan School for the Blind	18	8	26
4. Division of Services for the Blind	8	10	18
5. Aid to the Blind	14	8	22
6. Detroit Sight Saving and Braille Classes	3	1	4
7. Vision Section, Michigan Department of Health	2	3	5
8. Office of Vocational Rehabilitation	0	0	0
9. Sight Saving Classes of four large cities	4	0	4
10. National Society for the Prevention of Blindness	0	0	0
11. Letters to hospital records librarians	14	11	25
12. Letters to ophthalmologists	9	7	16
13. Non-professional individuals aware of the study	2	0	2
Totals	109	62	171

number of reascertainments at each source as a rough guide of the progress of the study and the thoroughness of coverage of the survey. Each time a name appeared from a different source it was listed as a separate ascertainment although we are quite aware that sources (1) and (2) are not independent, and sources (11) and (12) are also overlapping. In fact, it is possible to interrelate most of the above sources with each other. Certainly no single source would be expected to reveal all cases of aniridia in the state, and any given aniridic would not have an equal probability of being ascertained from all given sources. As an example, one would not expect to find a 65-year-old aniridic's name in the School for the Blind files (source 3) nor would one expect to locate a 5-year-old individual's record in the files of the Aid to the Blind Division (source 5) since blind pensions are not available to children. It may be theoretically possible to assign each aniridic individual in the state of Michigan a separate probability value for being located at each of the 13 sources, based on sex, age, severity of disease, socio-economic status, place of residence, etc., to arrive at an overall estimate of the "probability of ascertainment." In practice we feel that such calculations are not justified because they are based on arbitrary assumptions.

Several definitions of terms used in this paper are necessary at this juncture. A "familial" case refers to an individual with aniridia who has an affected parent, while an "isolated" aniridic is one whose parents have normal eyes. An "isolated" case, then, may be the starting point for a "familial kindred" and if the isolated aniridic has affected offspring, these children, by the definition above, are "familial" cases.

Of the above mentioned 67 non-ascertained familial cases discovered only through family studies, 44 were deceased or living out of state (table 2) and were thus not expected to appear in the rosters of most sources listed above. In the majority of the remaining 23 "non-ascertained" Michigan familial cases

Table 2. Status of Aniridia Population on January 1, 1959

Classification of aniridics	Probands ascertained by sources listed in Table 1	Non-probands "discovered" only through family history	Total
Living in Michigan on January 1, 1959			
"Familial" cases	53	23	76
"Isolated" cases	40	0	40
"Illegitimate" cases	2	0	2
Sub-Total	95	23	118
Living out of state on January 1, 1959	6	22	28
Dead on January 1, 1959	5	22	27
Born in Michigan after January 1, 1959	3	0	3
Total	109	67	176

the relatives were able to supply a reason why such individuals were not located by our methods of ascertainment. Such reasons included living out of state during school years although born in Michigan, females married to "good providers" who did not require state aid, mild cases who have not presented complications requiring an ophthalmologist's care, clinical consultation outside Michigan or eye surgery in a non-Michigan hospital, etc.

For the frequency estimates essential to the derivation of mutation rates and selection constants, one must select a reference point in time. As of January 1, 1959, 118 of the total 176 were alive and residents of the state of Michigan. The status of the remaining 58 cases is revealed in table 2. Although the "out-of-state" and "deceased" patients do not enter into the calculation of the incidence of the disease, they are of value in the derivation of segregation ratios, fertility estimates, etc.

The family material is summarized in four figures. Fig. 1 contains 12 kindreds in which each affected person had one affected parent, or in the cases of older generations of deceased individuals, one parent was presumably affected. In other words, all aniridics in Fig. 1 would be classified as "familial" cases. Fig. 2 summarizes the histories of 17 "isolated" cases, all of whom were probands, and all of whom reproduced. Eleven of these 17 isolated aniridics had at least one aniridic descendent. These affected children (and grandchildren) in the generations following the original isolated aniridic were classified as "familial" cases according to the definition given above. Fig. 3 is composed of 30 abstracted pedigrees of isolated probands who did not reproduce. It should be pointed out that whenever an "isolated" case was found, as much information as possible was obtained on all the direct ancestors whom the informants could recall, as well as specific information pertaining to the eyes of living and deceased collateral relatives. It was felt that if an informant could recall the color of the eyes of the individual in question, that individual most probably did not have aniridia. Often photographs from the family album would give evidence of the presence of irides, particularly in deceased individuals and those living too far away to be examined. With one exception, in the histories of every living isolated aniridic in our study, surnames of the four grandparents and some infor-

mation pertaining to their eyes were obtained, and in many cases information pertaining to the eyes of the great-grandparents seemed reliable. There were two cases of illegitimate aniridics with unknown paternity who were left unclassified, as shown in Fig. 4. It is perhaps significant that both of these cases were born in the same village in which the largest aniridic family in the study resided. In one instance the proband's mother lived only a few blocks from the affected family. In the second case the mother is deceased, but her twin brother stated that she had known one of the affected males in Kindred 1699. Blood and saliva tests were carried out on the probands, the possible aniridic father, the mother in Kindred 5400, and the father named on the birth certificate in Kindred 4027. This latter man was excluded on the basis of the Rh tests. The possible aniridic father was not excluded in either case.

The total population of 176 aniridics was distributed among 61 kindreds, of which 54 had at least one affected member living in Michigan on January 1, 1959. The remaining seven kindreds contained single, isolated cases. Three of these probands were living in Ohio (1671, 5090, 6610), the fourth was a 43-year-old single female born in Michigan but maintaining residence in Chicago for many years (4813), the fifth and sixth were males born to normal parents during 1959 (7016, 7084), while the seventh was an aniridic male who died in October, 1958 at two years of age (7110).

Three of the kindreds (4816, 4952, 4955) were Negro, with five aniridics residing in Michigan. The percentage of Michigan Negro aniridics was 4.24, compared to a "non-white" population percentage in Michigan, as given in the 1950 census data, of 7.12. The remaining cases were Caucasian of European ancestry, including English, Irish, Scotch, Dutch, German, Polish, Lithuanian, Hungarian, Italian, French, Danish, Swedish, and Norwegian.

THE FREQUENCY OF ANIRIDIA

The civilian population of the lower peninsula of Michigan for January 1, 1959 (estimated from data supplied by the State Bureau of Vital Statistics) was 7,604,811, and the known living Michigan aniridia population on the same date was 118. This gives a *minimum* incidence figure of 1:64,448, which is higher than that reported for Denmark by Møllenbach (1947). He found that in 1944 there were 40 aniridics living in Denmark, with a total population of 3,844,000, or an incidence of 1:96,100. If a Poisson distribution is assumed, the standard errors may be calculated, giving comparative frequency estimates of $(1.55 \pm 0.14) \times 10^{-5}$ for Michigan, and $(1.04 \pm 0.16) \times 10^{-5}$ for Denmark. These frequencies differ significantly at the 5 per cent level by the chi-square test.

As shown in table 3, 41/118 or 35 percent of the Michigan aniridics living on January 1, 1959 were ascertained two or more times, while 23/118 or 19 percent were missed by our methods of locating cases and discovered only in the course of family studies. In this effort to compile a complete roster of aniridia for Michigan, the number of familial cases missed should be negligible, since it is only necessary to ascertain one proband per familial kindred to "discover" the remaining cases by family history. This may not be said of the isolated cases of

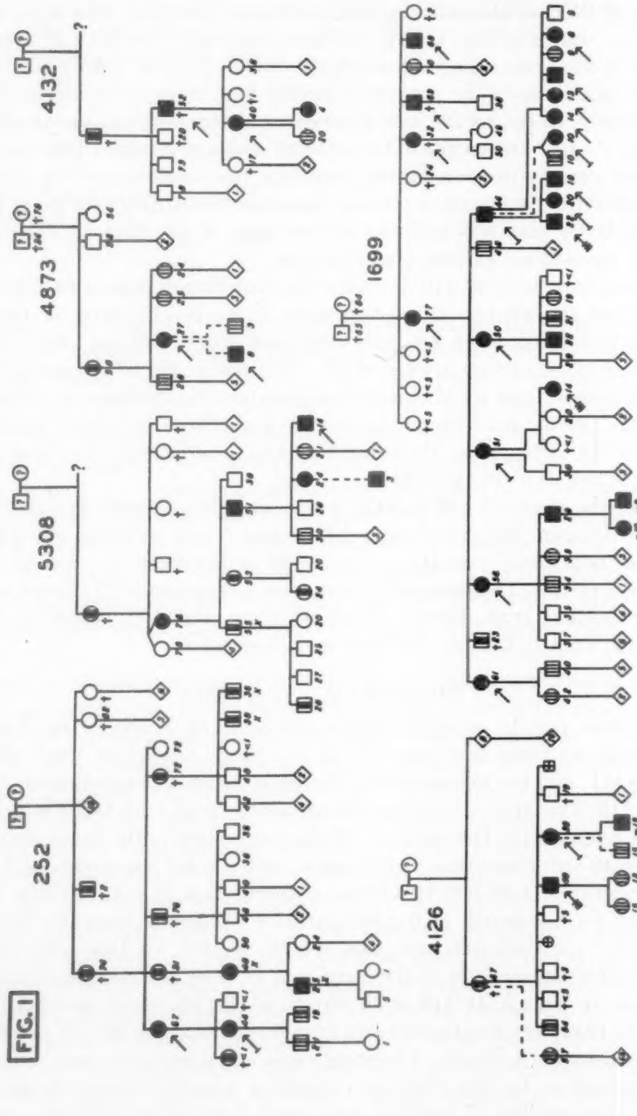
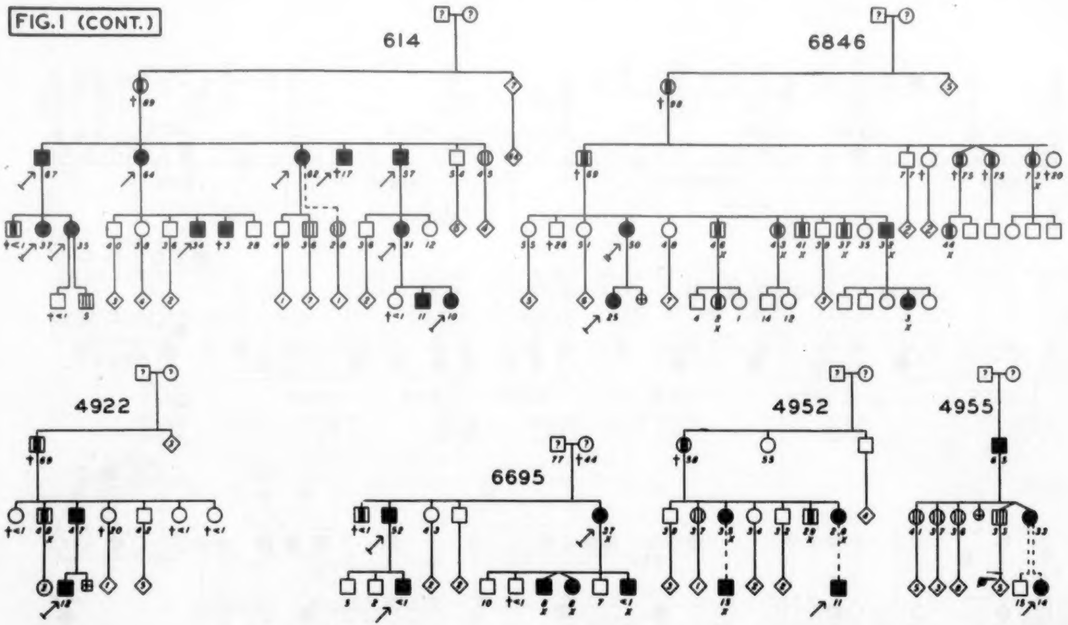


FIG. 1

FIG. I (CONT.)



One ascertainment
Two ascertainments
Three ascertainments
Four ascertainments

Male, reported normal
Female, reported normal
Examined, normal
Reported affected

Examined, affected
Sex not designated
Miscarriage
Stillbirth

† Dead on January 1, 1959
x Out of state on January 1, 1959
– Born after January 1, 1959

Digits inside symbols refer to number of individuals. Digits below symbols refer to age on January 1, 1959 or age at death. Digits above first sibship line refer to kindred number in files of Department of Human Genetics, University of Michigan, Ann Arbor.

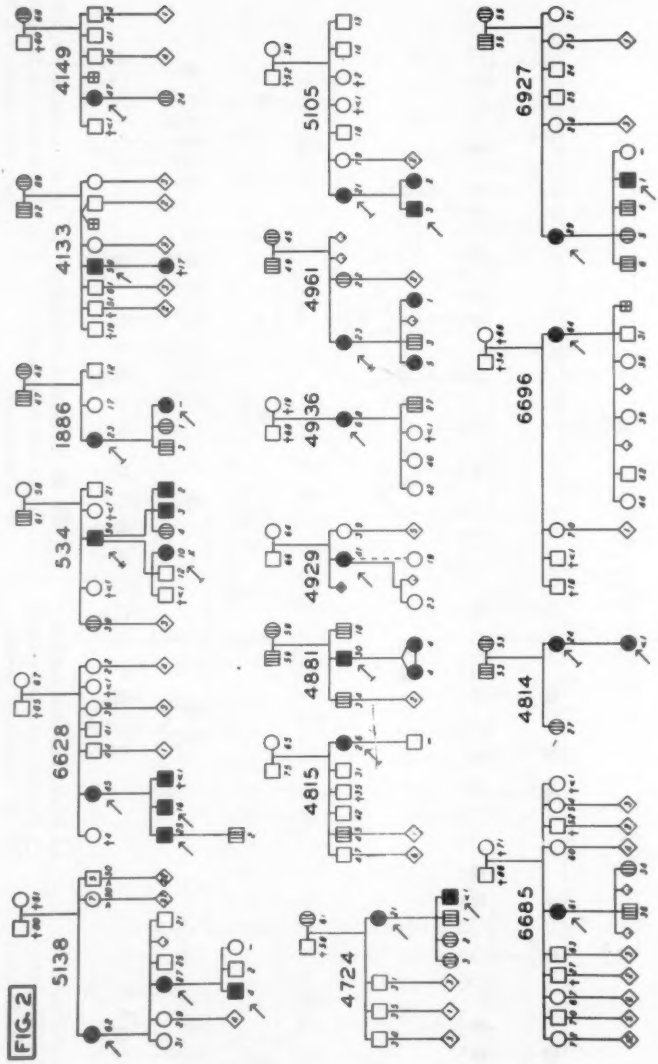

4149
4133
1886
534
6628
5138
5105
4961
4936
4929
4881
4815
4724
6927
6696
4814
6685

FIG. 2

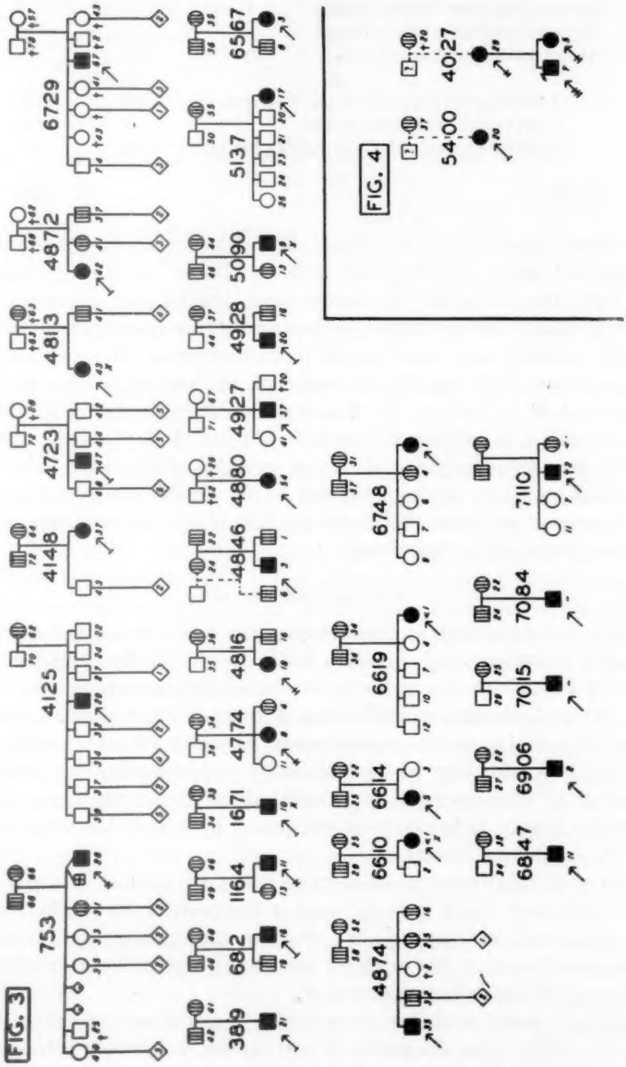


FIG. 3

FIG. 4

TABLE 3. FREQUENCY OF REASCERTAINMENT OF LIVING MICHIGAN ANIRIDICS

Ascertained from four sources	4		
Ascertained from three sources	8		
Ascertained from two sources	29		
Ascertained from two or more sources		41	35%
Ascertained from one source		54	46%
Ascertained only through family history		23	19%
Total		118	100%

aniridia where all relatives are normal. From the knowledge of the non-ascertained familial cases (23/76), and with the broad assumption that, by the methods used, the probability of ascertaining familial and isolated cases is approximately equal, then by simple proportionality, it is estimated that approximately 18 isolated cases have remained undiscovered. The roughness of this figure is obvious. Utilizing this estimate of 18 "missed" cases, the figure of 1:55,918, or $(1.79 \pm 0.15) \times 10^{-5}$, may be closer to the true value of the frequency of aniridia in Michigan than the value of $(1.55 \pm 0.14) \times 10^{-5}$ given above. The gene frequency would be just one-half of this value, since 1) there are few or no "carriers" of the gene who do not exhibit the aniridia phenotype, 2) we will present evidence that there are few, if any, phenocopies, and 3) the aniridic has a normal life expectancy.

CLINICAL DESCRIPTION

Following ascertainment of cases, field work was carried out to obtain detailed family histories, vital statistics, medical information, and fertility data. During these interviews arrangements were made for examinations of the aniridics, their parents, siblings, and offspring. A total of 122 eye examinations were performed on aniridics in the present study, of which 75 were made in the Department of Ophthalmology at the University of Michigan Medical Center and an additional 47 were reported by 24 ophthalmologists. Other cases of aniridia discovered by family histories, were confirmed by hospital records, by eye reports, or by physicians' letters.

The degree of manifestation of aniridia was very similar in the two eyes of the same individual. There were no cases in the present study where the condition was unilateral, with normal iris tissue in the opposite eye. When tags of iris tissue were found at the angle of the anterior chamber there was still no doubt that a gross iris defect was present.

Although not easily mistaken for aniridia, a few conditions should be mentioned in the differential diagnosis. These include coloboma, ectopia pupillae, primary atrophy of the iris, and Axenfeld's syndrome (see Duke-Elder, 1941; Falls, 1949; Heath, 1953). Coloboma iridis has been reported to occur in several aniridia pedigrees (e.g., Bell, 1932; Beattie, 1947), but this association was not found in Møllenbach's (1947) Danish survey, nor in the present Michigan study.

TABLE 4. COMPLICATIONS OF ANIRIDIA FOUND IN 122 EXAMINED CASES

Type of complication	Present	Absent	Not stated
Nystagmus	99	7	16
Cataracts	90	16	16
Glaucoma	54	36	32
Ectopia lentis	17	31	74

TABLE 5. VISUAL ACUITY OF ANIRIDICS IN THE BETTER EYE, CORRECTED VISION

Acuity as defined in text	Under 40 when tested	40 or over when tested
Fair, better eye	38	1
Poor, better eye	43	6
Blind, both eyes	7	9
Not stated	16	2

The frequency of certain common ocular complications found in our examinations are lasted in table 4. The visual acuity, as determined by our examinations, is summarized in table 5. Total blindness in both eyes, due to enucleation of the globe, degenerative changes of the cornea, mature cataracts, glaucoma, or phthisis bulbi, occurred in 16 of the 104 aniridics for whom there is accurate information. Seven of 88 individuals less than 40 years of age were blind, but 9 of 16 age 40 or over had no vision. The threat of total blindness thus increases with age. With reference to table 5, fair vision was defined as "between 20/60 and 20/200 corrected in the better eye" while poor vision referred to "light perception," "sees moving objects," "counts fingers," or "corrected vision of 20/200 or less in the better eye." It is apparent that almost all aniridics over the age of 40 have a severe visual handicap.

The "ultimate lesion" in aniridia is unknown. The iris develops relatively late in the embryonic period, and is not identified as a discrete organ on tissue sections until the 11th or 12th week of gestation, at the 70 or 80 mm. stage (Mann, 1937). It is composed of two layers. The superficial anterior layer is formed from mesoderm while the deeper posterior layer is derived from neurectodermal tissue at the rim of the optic cup. Two suggestions have been advanced concerning the mode of action of the gene. Some authors (e.g., Waardenburg, 1932; Sorsby, 1951) believe that defective neurectodermal tissue is the basis of aniridia and cite examples of aplasia of the macula associated with aniridia as evidence for this theory. As an alternative explanation, Mann (quoted in Bell, 1932) has suggested that at a critical stage in the development of the eye, the network of collagen fibers and embryonic blood vessels which surround the lens and attach to the filtering angle of the eye is not properly absorbed, thereby preventing forward and inward growth of the neurectodermal leaf of the iris. If the rim of the optic cup is held back by these structures, then the scaffolding on which the mesodermal leaf normally makes its attachment is lacking and the latter may fail to develop. Thus, the posterior layer of the iris lies curled back upon itself and is not stabilized in its natural position. Certain observations in

individuals with aniridia tend to lend credence to this second theory. For example, we have observed that when goniotomy is performed to correct glaucoma (a surgical procedure which entails a sweeping motion of the knife around the angle of the anterior chamber) occasionally pieces of iris tissue may be freed by this maneuver and fall into place. Also, the extent of the iris defect is somewhat variable and it is not unusual to observe tags of iris tissue beneath the corneoscleral junction, particularly if the angle is viewed through a gonioscope (François, 1955). In addition, Pincus (1948) observed that in all reported histopathologic examinations of aniridic eyes a small rim of iris tissue encircles the periphery of the anterior chamber and may be adherent to the cornea, obliterating the angle. It seems reasonable, then, to suggest that a certain amount of iris tissue is formed in aniridia and the absence of visible iris is a secondary manifestation of the action of the gene.

During the course of investigation, no systematic attempt was made to evaluate the physical and mental status of the individuals affected. However, certain congenital defects were reported during the collection of the family histories, in hospital records, by physicians' letters, or noted during the ocular examinations. Møllenbach (1947) observed "a strikingly great number of [aniridic] individuals of low intelligence. Only about one-third of the children were able to get along with ordinary school work. About one-third of the adult patients are inmates of institutions for public care while the remainder are occupied with occupations for the blind and the women are barely able to perform elementary housework." We have been unable to confirm Møllenbach's impression. Three aniridics were definitely mentally retarded but one had a history of meningitis at the age of three with residual deafness. The other two had intelligence quotients of 46 and 72. Five others appeared dull but psychometric evaluations were not obtained and the problem of evaluating intelligence in the visually handicapped is well known. Of these five, two were reported by pediatricians to have possible mental retardation; one had a history of convulsions and was in the third grade at age ten; another had a speech defect and one examiner believed the mentality of the patient at age 15 was not beyond the fourth grade level; and the last, an adult female, was reported by the family members and the social worker to be mentally retarded. Not included in the enumerations above were a Mongolian idiot (6906) and a male who died at age two with hydrocephaly and associated retarded development (7110). Two cases of hypospadius and three instances of bilateral cryptorchidism were confirmed by examination. It should be emphasized that these findings are incidental to our eye examinations and we are unable to evaluate the true frequency of associated congenital malformations. However, these male genital anomalies may be related to our findings of decreased male fertility to be reported below.

The individual with aniridia finds himself at a social as well as a physical disadvantage. This is reflected in his need for special schooling and special employment. Twenty-seven of the 95 Michigan aniridics age 6 and over were known to have attended the State School for the Blind, while eight more have attended sight saving classes in public schools. More than one-half of the

aniridics over age 20 have received financial assistance or special services from the state. A more important social disadvantage which has biological implications is the aniridic's decreased success in securing a spouse. This will be discussed more fully under relative fertility.

SURVEY OF THE "FAMILIAL" CASES OF ANIRIDIA

There were 118 aniridics living in Michigan on January 1, 1959, and 76 of these individuals had an affected parent. These 76 "familial" cases fall into 24 kindreds (Figs. 1, 2, and 4). There were 35 males and 41 females, which is not a significant departure from a 1:1 sex ratio ($\chi^2 = 0.47$; $0.50 > P > 0.40$). In two kindreds there was a fairly reliable history of parents with normal appearing eyes and no visual problems, who reproduced more than one affected offspring. One of these (1699) involved a sibship of four affected and six normal offspring and has been discussed in detail in a previous publication by Reed and Falls (1955). Gonadal mosaicism has been offered as one explanation for this pedigree. Nonpenetrance seems to be an unlikely explanation since there is no evidence for it elsewhere, either in the present study or in reports by other investigators. In the second family (6695), the father is age 74, living in the state of New York, and unavailable for examination, whereas the mother died in 1928, but was reported by her husband and several other members of the family to have normal blue irides and normal visual acuity. This couple was naturally concerned when three of their five children were born with severe visual handicaps. They corresponded with relatives in Denmark, but could find no cases of aniridia in past or contemporary generations. One of the affected children died in infancy, but the other two have reproduced aniridic offspring. This situation has not arisen in any of our "younger" kindreds where both parents are living and available for examination.

There were no pedigrees in our series which revealed "skipping a generation." Reed and Falls (1955) tabulated 63 cases of individuals with an aniridic parent and aniridic offspring previously reported in the literature. (From the present series, 27 more cases can be added to their list.) In only five instances was there a question of a non-aniridic intervening between affected parent and affected child. In two of the reports there were iris irregularities of sufficient degree to presume the person in question actually carried the gene. In the other three cases the descriptions were inadequate to draw conclusions as to lack of penetrance. In none of these five unusual cases were all three generations ophthalmologically examined.

There were three individuals in three separate kindreds in the present study in which the usual phenotype was incompletely manifested. In one case (1699), examination revealed hypoplasia of the iris stroma, irregular pupillary margins, nystagmus, photophobia, and decreased visual acuity. In another case (614), the pupils were described as enlarged and egg-shaped or ovoid with definite iris tissue visible. Neither of these two individuals has reached reproductive age. The third person who demonstrated "incomplete aniridia" (5308) had an

aniridic mother, two aniridic sibs and two aniridic offspring. His eyes were carefully examined and the following findings recorded:

Right eye: anterior chamber of normal depth, defective mesodermal leaf of iris from 6:30 to 10 o'clock, clockwise, with baring of underlying neurectoderm; iridoschisis at 8 o'clock.

Left eye: iris pupillary border incompletely developed, temporal iridoschisis from 2 to 9:30 o'clock, clockwise; neural ectoderm seen through the atrophic dehiscent mesodermal leaf of iris.

With a moderate amount of pigmented neurectodermal iris tissue visible to the examiner, it is perhaps significant that this man considered himself to have aniridia like his affected relatives; for this reason he was eager to cooperate in our ophthalmological examinations. It is worth reiterating here that the degree of incompleteness of expression was approximately equal bilaterally in each of these three cases and the phenomenon of unilateral aniridia with normal iris tissue in the opposite eye has not been observed.

In summary, it may be concluded that there were no cases of aniridia in our familial groups which did not behave according to expectations for a condition caused by an autosomal dominant gene with complete penetrance and little variability in expression.

SURVEY OF THE "ISOLATED" CASES OF ANIRIDIA

There were 40 aniridic probands living in Michigan on January 1, 1959, who were found to have normal parents with respect to the aniridia trait. Seventeen of these 40 isolated cases have reproduced (Fig. 2). Of the remaining 23 (Fig. 3), 12 were under age 18 and 11 were age 18 or over and single. There were 16 males and 24 females; a non-significant excess of females. Detailed pedigrees were assembled on these 40 cases following careful histories obtained on as many individuals as feasible and in not a single instance was an affected parent, grandparent, great-grandparent, sib, or collateral relative found. Eleven of the 17 isolated cases who reproduced had at least one affected child. The isolated parent in these 11 kindreds almost certainly owes his disease to a dominant mutation. Among the remaining six individuals, two had a single child, two others had two children each, one had four offspring, and one had five. In addition to these 15 livebirths, these latter six women reported five miscarriages and one stillbirth (table 6).

Consanguinity between the parents of the isolated cases was not discovered. This fact plus the lack of clear demonstration of normal parents with two or more affected children rules against autosomal recessive inheritance. Sex-linked recessive inheritance is excluded by the sex ratio. This observation of six isolated cases who reproduced only normal children may thus most likely be attributed either to the fact that the isolated aniridic parents were phenocopies or to the vagaries of genetic segregation. (Only two reproductive histories among these six fertile isolated aniridics deviate to a degree that the possibility of non-genetic aniridia arises as a reasonable explanation.) By extrapolation, on the exaggerated assumption that six of 17 fertile isolated cases, or 35.3

TABLE 6. OFFSPRING OF 17 FERTILE ISOLATED ANIRIDICS

	Kindred no.	Sex of parent	Normal male	Normal female	Aniridic male	Aniridic female	Remarks
1	534	♂	2	1	2	1	One normal male died age 1 mo. of "malnutrition."
2	1886	♀	1	1		1	
3	4133	♂				1	Affected female died age 17 following thyroid surgery.
4	4149	♀		1			
5	4724	♀	2	1	1		
6	4814	♀				1	
7	4815	♀	1				
8	4881	♂				1	Identical twins.
9	4929	♀		2			One miscarriage.
10	4936	♀	1	3			One normal female died age 10 mos. of "meningitis."
11	4961	♀	1			2	One miscarriage.
12	5015	♀			1	1	
13	5138	♀	2	2		1	One miscarriage.
14	6628	♀			3		One affected male died age 5 days of hydrocephalus.
15	6685	♀	1	1			Two miscarriages.
16	6696	♀	2	3			Two miscarriages; one still-birth.
17	6927	♀	2	2	1		
Totals			15	17	8	9	

percent, are phenocopies, the estimated maximum number of phenocopies among the 40 isolated cases is approximately 14. Reasons for doubting that there actually are phenocopies will be developed later. Accordingly, it will be assumed that at least 65 percent—and more likely close to 100 percent—of isolated cases of aniridia are due to dominant mutation.

Several environmental factors have been investigated for a possible relationship to the appearance of isolated cases, i.e., to the mutational process. These include season of conception, effect of birth rank, parental age effect, and history of maternal illness during early pregnancy.

1) The months of conception of isolated cases were fairly evenly distributed in the 36 cases for which the birthdates were known, from January to December, as follows: 2, 2, 3, 5, 2, 7, 3, 2, 1, 5, 1, and 3. Grouping these cases by three-month intervals, there is no significant departure from a random distribution, although it should be remarked that the second quarter of the year contributes 14 of the 36 cases.

2) The effect of birth rank on the appearance of isolated cases was tested by the method of Haldane and Smith (1948) and is presented in table 7. The difference between observed and expected (558–525) is less than one standard error.

3) Since the mean birth year of the isolated cases was 1928 and the median year was 1932, the census year 1930 was chosen to compare the age of mother in the general population with age of mother at birth of isolated cases. Penrose

TABLE 7. EFFECT OF BIRTH RANK AMONG ISOLATED CASES
(Method of Haldane and Smith, 1948)

Sibship size	Birth rank 1	2	3	4	5	6	7	8	9	10	Total
2	6	5									11
3	2	4									6
4		1		2							3
5	1	1	1		2						5
6						3					3
7	1	1		1	1						4
8					1						1
9											0
10						1					1
Total	10	12	1	3	4	4					34*

$6A = 558$
$m = 525$
$V = 2115$
$\sigma = 45.88$

* Birth rank unknown in one sibship and single offspring in four sibships. One individual (6927) was not included; she was first-born in a sibship of six.

TABLE 8. MATERNAL AGE EFFECT

Mother's age at birth of isolated case	Observed number of isolated cases	Expected number of isolated cases*
15–19	7	4.7
20–24	7	11.5
25–29	7	9.3
30–34	9	6.2
35–39	6	3.8
40–44	1	1.3
45+	0	0.1
Total	37**	
$\bar{x}$	27.3 years	26.6 years

* Expected values are derived from maternal age data given by the Bureau of Vital Statistics for United States, 1930, assuming no maternal age effect.
** Age of mother unknown in two cases; kindred 6927 not included.

(1955), in a review of data from several mutation rate studies, has called attention to a "parental age effect" amounting, on the average, to just over one year. As shown in table 8, the observed mean age of the mother of isolated aniridics is 0.7 years greater than expected, which is in keeping with the results of similar studies. The average difference in husband-wife age in 1930 was 4.6 years (Glick, 1957). The mean difference in fathers' and mothers' ages in the isolated cases was 3.2 ± 0.6 years. Therefore, we are unable to show a paternal age effect.

4) An abnormal pregnancy history was a very unusual feature in the general information provided by the mothers of the isolated cases. In two instances

there was a history of uterine bleeding during the first trimester. No mothers reported unusual complications of pregnancy such as toxemia, hydramnios, or placenta praevia while carrying the aniridic fetus and there were no histories of unusual parental radiation exposure.

MUTATION RATE

Mutation rate estimates can only be as accurate as the completeness of ascertainment. For this reason, it was felt worthwhile to compare the age spread of the aniridia population with that of the general population of Michigan to determine whether there were any glaring deficiencies in ascertainment for any particular age group. In the case of aniridia, since older individuals tend to come to medical attention because of the complications of their disease, or to state attention because of benefits to the blind, then young affected individuals should be missed more frequently. Conversely, if the life expectancy of affected individuals is less than that of unaffected persons, then a decrease in the proportion of aniridics in the older age group would be expected. Fig. 5 compares the age distribution of the general population of Michigan with that of our known aniridia population. There is no evidence of a deficiency in any particular age group. While it seems unlikely that all cases of this disease in the state have been located, there is, on the other hand, no hint that a disproportionate number of young affected individuals has been missed.

A direct estimate of the rate with which mutation results in the aniridia phenotype may be obtained in several ways. One approach utilizes the number of known isolated aniridics born in a specified time interval compared to the total number of livebirths in the state during the same time span. For the 40-year period from January 1, 1919 to January 1, 1959, the estimate obtained is

$$\frac{28}{4{,}664{,}799} \times \frac{1}{2} = (3.0 \pm 0.8) \times 10^{-6} \text{ mutations/locus/generation} \qquad (1)$$

Another estimate can be obtained from the number of isolated aniridics alive on a certain date and the total population. This, of course, assumes that there is no selective loss of aniridics due to a relatively decreased life ex-

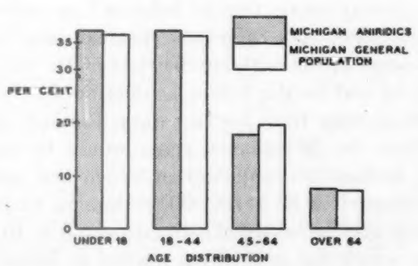


FIG. 5

pectancy. This is felt to be a permissible inference in view of the findings charted in Fig. 5. For the date January 1, 1959, a rate of

$$\frac{40}{7,604,811} \times \frac{1}{2} = (2.6 \pm 0.6) \times 10^{-6} \qquad (2)$$

is obtained, which agrees, within error due to chance, with the estimate given in equation (1).

If allowance is made for the possibility of non-genetic aniridia, then somewhat lower estimates result. In accord with the discussion of a *maximum* proportion of phenocopies in the previous section of 35.3 per cent, a reduction in the mutation rates given in equations (1) and (2) will yield lowered estimates of 1.9×10^{-6} and 1.7×10^{-6} respectively. We regard this as an overcorrection.

If isolated cases were missed by our methods of locating patients, then the above estimates are too low. By definition, all of the isolated cases are probands. However, as mentioned previously, 23 of the 76 familial cases, or 30.3 percent, were discovered by family history alone, giving a rough estimate of 18 isolated cases missed. If we apportion 13.3 of these 18 "missed" isolated cases to the group born in the 40-year period under consideration for the mutation rate estimate in equation (1) (on the basis of the birth dates of the "missed" or non-proband familial cases), then corrected values of $(4.4 \pm 0.7) \times 10^{-6}$ and $(3.8 \pm 0.5) \times 10^{-6}$, respectively, are obtained. We regard these values as the best approximation to the "true" rate. Again, it should be emphasized that this is an accurate locus rate only if mutations resulting in the aniridia phenotype are restricted to a single locus and all mutations at this locus result in that phenotype.

The only other available estimate for the mutation rate of the aniridia gene is given by Møllenbach's data for Denmark (1947). From his survey of records of seven hospitals, eye clinics, and blind schools, as well as through correspondence with Danish physicians, he located 28 "primary cases" born in the 70-year period from 1875 through 1944, in a total of 4,809,746 livebirths during the same period. Calculating a mutation rate directly from his data, $(2.9 \pm 0.8) \times 10^{-6}$ is obtained, which is in complete agreement with the Michigan rate derived by the same method and given in equation (1) above. However, Møllenbach emphasizes that he believes cases were missed because of high infant mortality rate, lack of proper medical care, and failure to keep complete records in some of the outlying districts of Denmark during the latter part of the last century and for this reason he does not believe the mutation rate should be estimated directly from his raw data. Instead, he assumes that the estimate derived from the 28 primary cases would be approximately 33 to 50 percent too low, so that the frequency at which new mutants occur should "more likely be estimated to be 1:100,000, reckoning with a suitable margin of safety." This would give an adjusted estimate of 5.0×10^{-6} mutations/gene/generation, a figure which has often been quoted in human genetic literature (Neel and Schull, 1954; Reed and Falls, 1955; Penrose, 1955).

This estimated mutation rate of the locus for congenital aniridia of ap-

proximately four per million gametes is one of the lowest reported for human genes. Considering the ease of identification of the phenotype for the aniridia gene and the fact that it is present at birth, fairly constant in expression, and exhibits high penetrance, it is one of the most satisfying conditions which could have been chosen for a genetic study. The issues at hand were not confused and complicated by incomplete pentrance, late onset, early death, and difficulties in diagnosis which have harrassed investigators dealing with other human traits.

RELATIVE FITNESS

The fertility of aniridics was estimated by comparing the number of their offspring with that of two control groups. These groups were (1) siblings of isolated aniridics, and (2) females in the general population of Michigan. It was found that the fitness of the aniridics did not differ significantly from either of these two groups. However, the control groups did differ significantly from each other. The control sibs were more fertile than the general population controls, while the aniridics occupied an intermediate position. The data on

TABLE 9. RELATIVE FITNESS OF ANIRIDICS BORN BEFORE JAN. 1, 1919 COMPARED TO SIBS OF ISOLATED CASES

	No. of aniridics born before 1-1-1919	No. of aniridics who reproduced	Total number of livebirths	Mean number of livebirths	Relative fitness of the aniridic individual	No. of aniridic livebirths	Per cent of aniridic livebirths	Mean number of aniridic livebirths	Relative fitness of the aniridia gene
	a	b	c	d	e	f	g	h	i
Male familials	18	12	43	2.39	0.81	15	34.9	0.83	0.56
Female familials	22	20	68	3.09	1.05	29	42.6	1.32	0.90
Male isolated cases	3	1	1	0.33	0.11	1	(100.0)	0.33	0.22
Female isolated cases	9	7	26	2.89	0.98	5	19.2	0.56	0.38
All males	21	13	44	2.10 ±0.52	0.71 ±0.20	16	36.4	0.76 ±0.38	0.52 ±0.26
All females	31	27	94	3.03 ±0.35	1.03 ±0.17	34	36.2	1.10 ±0.25	0.75 ±0.17
All familials	40	32	111	2.78 ±0.35	0.95 ±0.17	44	40.0	1.10 ±0.27	0.75 ±0.18
All isolated cases	12	8	27	2.25 ±0.58	0.77 ±0.22	6	22.2	0.50 ±0.25	0.34 ±0.17*
All Aniridics	52	40	138	2.65 ±0.30	0.90 ±0.15	50	36.2	0.96 ±0.22	0.65 ±0.17*

* Deviation from 1 > 2 S.E.

relative fitness of aniridics when compared to siblings of isolated cases are summarized in table 9. Several limitations should be noted here:

(1) Only aniridics born before January 1, 1919 were counted. These included both familial and isolated cases and included those who died and those who remained single as well as those who reached reproductive age, married, and proved themselves fertile. The year 1919 was chosen because these individuals would have reached their fortieth birthday or would have died prior to age 40 and their reproduction could be considered essentially complete.

(2) Those aniridics were excluded who were "discovered" because of their fertility, i.e., ascertained only through a child.

(3) All siblings of isolated cases born before 1919 were included regardless of viability, marital status, or fertility.

(4) Only liveborn offspring were considered.

Unaffected siblings of familial aniridics were not used for control purposes because it was considered possible that they might have deliberately limited their reproductivity. It has been shown by Reed and Neel (1959) in a study of Huntington's chorea that normal siblings of familial choreics had a lower reproductive performance than the choreics themselves, but that the latter in turn were less fertile than the general population.

There were 159 livebirths to 54 normal sibs of isolated cases, or 2.94 ± 0.36 mean livebirths per sib. As shown in table 9, column e, the overall relative fertility of aniridics when compared to these sibs is 0.90 ± 0.15. The fitness of the aniridic female is 1.03 ± 0.17 and of the male, 0.71 ± 0.20. This difference between male and female reproductive performance is not statistically significant. However, a difference in the same direction and of approximately the same magnitude has been noted in at least two other dominantly inherited diseases, neurofibromatosis (Crowe, Schull, and Neel, 1956) and Huntington's chorea (Reed and Neel, 1959), suggesting the generalization that in our present culture the male with a dominantly inherited disease of any severity is at a greater reproductive disadvantage than the female. In the present instance it is not clear to what extent the reduced fertility is biologically determined, as suggested earlier by the occasional findings of associated hypospadius and cryptorchidism, and to what extent sociologically determined, as reflected in the lower marriage rate of aniridic males.

In this latter connection, the overall marriage rate of Michigan aniridics living on January 1, 1959 who had reached age 20 is 50/70 or 71.4 percent. Table 10 reveals that a decreased marriage rate occurs in both sexes but is much more marked in the male, when compared to the general population. It

TABLE 10. COMPARATIVE MARRIAGE RATES

Sex	All persons over age 14 (Michigan census data, 1950)	Aniridics over age 14 (living in Michigan in 1959)	Persons blind from any cause (Franceschetti, 1935) (German statistics data)
Males	0.75	0.50	0.47
Females	0.81	0.72	0.72

TABLE 11. RELATIVE FITNESS OF ANIRIDICS AGE 40 AND OVER COMPARED TO GENERAL POPULATION†

	Number of aniridics who reached age 40	Number of aniridics who reproduced	Total number of live births	Mean number of livebirths	Relative fitness of the aniridic individual	Number of aniridic livebirths	Per cent of aniridic livebirths	Mean number of aniridic livebirths	Relative fitness of the aniridia gene
	a	b	c	d	e	f	g	h	i
Male familials	14	12	43	3.07	1.20	15	34.9	1.07	0.84
Female familials	19	18	65	3.42	1.34	28	43.1	1.47	1.15
Male isolated cases	3	1	1	0.33	0.13	1	(100.0)	0.33	0.26
Female isolated cases	9	7	26	2.89	1.13	5	19.2	0.56	0.44
All males	17	13	44	2.59 ±0.58	1.01 ±0.23	16	36.4	0.94 ±0.45	0.73 ±0.35
All females	28	25	91	3.25 ±0.36	1.27 ±0.14	33	36.3	1.18 ±0.28	0.92 ±0.22
All familials	33	30	108	3.27 ±0.36	1.28 ±0.14	43	39.8	1.30 ±0.31	1.02 ±0.24
All isolated cases	12	8	27	2.25 ±0.58	0.88 ±0.23	6	22.2	0.50 ±0.25	0.39 ±0.20*
All aniridics	45	38	135	3.00 ±0.32	1.17 ±0.13	49	36.3	1.09 ±0.24	0.85 ±0.19

† General population fertility taken from data of U.S. Census, 1950.
* Deviation from 1 > 2 S.E.

is interesting to note that the marriage rate of aniridics is almost identical with that reported by Franceschetti (1935) for all blind individuals residing in Germany. This would suggest that the decreased marriage rate is related to loss of vision per se, and not a unique reduction in fitness attributable to the aniridia gene. Severe visual handicap from any cause will hamper the male's success more than the female's in finding a spouse.

Utilizing the second control series, an estimate was obtained of the relative fertility of aniridics as compared to Michigan females. These data are presented in table 11. Again, it is necessary to note the following restrictions applied to the data:

(1) Only aniridics who reached their fortieth birthday were included. This was necessary because their fertility is compared to that of females in the general population who attain a given age as reported in the census data. From a biological point of view, this is a severe limitation because any reduced fertility due to reduced viability will not be apparent.

(2) All females in the general population of Michigan who reached age 40 were included, regardless of their marital status.

(3) Census fertility reports contain data for females only. For any large interbreeding population with a 1:1 sex ratio the fertility of all males compared to all females must be equal. However, for small subgroups where mating can occur outside the group, this truism does not necessarily apply.

(4) The standard errors in the census data are negligible compared with the magnitude of variance in our sample and are not considered when making comparisons.

The 1950 census returns yield a value of 2.56 mean livebirths for Michigan females age 40–59. The aniridics who reached age 40 have a fitness of 1.17 ± 0.13 when compared to these controls (table 11, column e). Again, the sex difference is noted, with a female fitness of 1.27 ± 0.14 compared to that of the male of 1.01 ± 0.23.

Finally, a comparison should be made between the two controls groups. It is not possible to compare them directly unless only the sib controls who reached age 40 are considered. There were 157 live babies born to 45 sibs who reached age 40, giving a mean number of livebirths per sib of 3.49 ± 0.34. Compared to the census figures of 2.56 mean livebirths per Michigan female as given above, the sibs of isolated cases have a relative fitness of 1.36 ± 0.13. This fitness value is a departure from unity by nearly three standard errors and raises the issue of whether even siblings of isolated cases may be used as "controls" since they do not reflect the fertility of the general population, at least in the present study.

GENETIC RATIOS

Although there is no doubt that aniridia is due to a dominant gene whose penetrance is close to 100 per cent, a variety of approaches suggest a significant departure from a 1:1 ratio in segregating sibships. If we restrict our attention to those kindreds which contain familial cases only (Fig. 1), then in 59 sibships in 12 kindreds there are 95 aniridic and 132 normal individuals (0.42:0.58), a departure from a 1:1 ratio which is significant at the .05 level. This treatment makes no allowance for the fact that in each kindred, ascertainment has been through one *or more* affected individuals. Accordingly, any correction for ascertainment can only increase the departure from a 1:1 ratio. Now, the statistical problems involved in correcting for the bias introduced by multiple ascertainments in multiple generations, the situation which exists in the present case, are of a rare degree of complexity and entail some rather arbitrary decisions concerning ascertainment probability which we are unwilling to make in view of the actual situation which obtains for these data, as discussed in the opening section. There are, however, some approximation procedures which entail minimum assumptions and corrections.

The simplest of these is to omit from any calculation of ratios the index case in each kindred. If one proband is deleted from each of the 12 familial kindreds in Fig. 1, then a ratio of 83 affected to 132 normal is obtained (0.39:0.61). This simple, but straightforward, correction for ascertainment further distorts the ratio, to a significance level of .001.

A second approximation to defining the true genetic ratio is to base the calculation of ratios on the offspring of each individual who served as a proband. This approach is perhaps the least biased of any method but sacrifices more than one-half of the data. If only offspring of probands in Fig. 1 are taken into consideration, there are 37 affected children and 47 normal children in 24 sibships (0.44:0.56). This is not a significant deviation from the 1:1 ratio ($P > .20$).

The treatment of the kindreds summarized in Fig. 2 presents a problem. As noted above, these 17 kindreds include 6 isolated cases of aniridia who were not proved by the progeny test to be mutants. Each of the remaining 11 kindreds was ascertained through the isolated affected parent and in addition, sometimes through an affected child as well. Since for all 17 kindreds ascertainment is through an affected parent, it seems legitimate to consider the ratio of normal to affected among their offspring with no further correction of the ratio for the additional ascertainments. There were 17 aniridic and 32 normal offspring of all 17 isolated cases who reproduced (Fig. 2), or a ratio of 0.35:0.65, the probability of this departure from a 1:1 ratio having a P value below 0.05. However, if we assume an expected proportion of 0.39:0.61, on the basis of the data derived from the familial cases above, it can be shown that this distribution of 17:32 fits quite well ($\chi^2 = 0.31$; $.60 > P > .50$). There are only 6 individuals in the present study on which an argument for the occurrence of phenocopies may be based. In view of the evidence that the ratio of normal to affected, when the offspring of these 6 are included with the offspring of proven genetic isolated cases, does not differ significantly from the ratio observed in the offspring of familial cases, we feel that the burden of proof is on him who maintains that any of these 6 individuals represents a phenocopy.

When all familial and isolated cases are considered together, with the *minimum* correction for ascertainment introduced by subtracting one propositus from each familial kindred, then a ratio of 100 affected to 164 normal is obtained (0.38:0.62), a departure from equality which is significant at the fiducial limit of 0.0001. We consider this value the best working estimate of the at-birth ratio to be derived from the present data.

Similar abnormal ratios have been obtained in other studies of aniridia. Beattie (1947) reported a large English pedigree with 29 affected and 44 normal (0.40:0.60). Paganelli (1951) found 41.8 per cent affected offspring in 74 sibships in 28 kindreds in a literature survey. In neither case was the information submitted sufficient to permit allowance for ascertainment. Grove, Shaw, and Bourque (1960) reported a large French Canadian kindred with 76 aniridics and 88 normals (0.46:0.54). This kindred was subject to multiple ascertainments and the best method of allowing for ascertainment bias is not clear. The number of affected individuals required to stimulate a geneticist's interest in a single pedigree depends on numerous, complex, known and unknown factors. In the Canadian kindred there were 21 cases reported in a single listing supplied by the Canadian National Institute for the Blind before the survey was undertaken, but subtracting 21 probands would be an obvious overcorrection. The data from

the Canadian and English pedigrees together with Møllenbach's (1947) eight Danish familial kindreds in which propositi are not designated are combined with the Michigan data in table 12. With *no* correction for ascertainment whatsoever, the segregation ratio in the combined studies stands at 239 affected to 317 normal (0.43:0.57), ($\chi^2 = 10.94$; $P < .001$).

Several causes for the abnormal ratios have been excluded. The deficiency of affected offspring is not related to the sex of the parent. In the Michigan survey, there were 21 aniridic males who produced 36 affected and 59 normal offspring (0.38:0.62), while 38 affected mothers had 59 affected and 73 normal children (0.45:0.55). The heterogeneity chi-square value for these two ratios is equal to 1.02, with a P value greater than .30. The explanation does not lie in the fact that one or two large kindreds are contributing to the abnormal ratio while the segregation in the other kindreds is normal. It is shown in table 13 that among 12 familial kindreds in Fig. 1 the genetic ratios were found to deviate in

TABLE 12. COMPARISON OF SEGREGATION RATIOS IN ANIRIDIA SURVEYS, WITH NO CORRECTIONS FOR ASCERTAINMENT

Locality	Number of kindreds	Number of sibships	Normal offspring	Affected offspring	Per cent affected	χ^2	P	Source
Michigan	24	76	164	112	40.6	9.80	<.005	Shaw, Falls, & Neel, 1960
England	1	11	44	29	39.7	3.08	.10–.05	Beattie, 1947
Canada	1	28	88	76	46.3	0.88	.40–.30	Grove, Shaw, & Bourque, 1960
Denmark	8	10	21	22	53.7	0.02	.90–.80	Møllenbach, 1947
Total	34	125	317	239	43.0	10.94	<.001	
(Survey of literature)	28	74	?	?	41.8			Paganelli, 1951

TABLE 13. GENETIC RATIOS IN SEGREGATING SIBSHIPS IN MICHIGAN "FAMILIAL" KINDREDS IN FIGURE 1*

Kindred number	Normal	Aniridic
252	15	9
614	14	12
1699	25	23
4126	8	2
4132	7	2
4873	4	1
4922	8	2
4952	4	4
4955	5	1
5308	13	8
6695	7	6
6846	22	13

* One proband deleted from each kindred.

TABLE 14. GENETIC RATIOS BY SIBSHIP SIZE

Size of sibship†	Number of sibships	Affected	Normal	Proportion affected	χ^2 (Yates' correction)
1	8	6	2	0.75	1.13
2	15	9	21	0.30	4.03*
3	9	10	17	0.37	1.33
4	6	8	16	0.33	1.96
5	9	16	29	0.36	3.20
6	3	6	12	0.33	1.39
7	6	22	20	0.52	0.02
8	0				
9	0				
10	1	4	6	0.40	0.10
11	1	8	3	0.73	1.45
12	1	6	6	0.50	0
Total	59	95	132	0.42	6.03*

* Significant at 0.05 level.
† Half-sibs were included in total sibship count.

the same direction in each case. Genetic ratios enumerated by sibship size may be found in table 14. There is no consistent trend toward the expected 1:1 ratio as the size of the sibship changes. As stated above, the ratio in the offspring of 24 probands was 37:47 (0.44:0.56). This may be compared to 35 non-probands in whom the offspring ratio was found to be 58:85 (0.41:0.59). These two ratios do not differ significantly from each other, suggesting that non-proband histories were probably as reliable as those of probands. Finally, in searching for clues as to the cause of these aberrant ratios, the information on individuals who died in infancy was examined to determine if inaccurate reporting of the condition of the eyes could account for the findings. Twelve infants who died under one year of age were reported to have normal eyes compared to four deceased infants reported to be aniridic. It seems unlikely, then, that errors in memory of presence or absence of the trait in deceased infants could account for more than about four cases.

If the departure from a 1:1 ratio is accepted as significant, several possible explanations must be considered. The first is "prezygotic" selection, encompassing such diverse situations as abnormal meiotic ratios, differential gametic survival, differential sperm motility, or sperm selection at the time of insemination or fertilization. Since the aberrant ratios appear in the offspring of both sexes, the latter two possibilities cannot be the sole factors. A second explanation is "postzygotic" selection. The developing zygote heterozygous for the aniridia gene could have a decreased viability. In the latter case, it might be possible to detect this by a history of excessive miscarriages. A tabulation of the miscarriages reported by isolated cases may be found in table 6; the frequency does not seem to be unusually high. Miscarriage data were obtained on these cases as carefully as possible whereas such detailed abortion histories in the familial kindreds were not so carefully sought and are not presented here because of incomplete information. Non-penetrance and incomplete expression as a cause for the abnormal ratios have already been dismissed.

Since the data in tables 9 and 11 suggest that aniridic individuals produce as many live offspring as normal individuals (column e), but that somewhat less than half of the offspring are affected (column g), it is not sufficient, in considerations of population dynamics, to confine comparison of the affected and normal individuals in the population to relative reproductive fitness, per se. In addition, the fitness of the *aniridia gene* in the present instance should be compared with that of its normal allele. This "gene fitness" may be derived directly from the data, and will include both "prenatal fitness" as well as "phenotypic fitness" or biologic fitness of the individual who appears with the trait.

Turning to table 9 (columns a and f), 52 aniridics produced 50 affected children. The mean number of affected offspring per affected parent is 0.96 $\pm$ 0.22 (column h). The control sib population produced 159 offspring, giving a mean number of normal offspring per normal parent of $159/54/2 = 1.47 \pm 0.18$. Assigning a relative fitness of one to the normal allele, the direct relative fitness of the aniridia gene when compared to this normal allele found in the sibs of isolated cases is estimated to be $(0.96 \pm 0.22)/(1.47 \pm 0.18)$, or 0.65 ± 0.17 (column i). By the same token, the fitness of the gene when compared to the normal allele in the general population is 0.85 ± 0.19 (table 11, column i).

It may be concluded, then, that the proportion of affected offspring of aniridic individuals, regardless of the sex of the parent, is about 0.38 instead of the expected Mendelian proportion of 0.50, and although persons with aniridia may expect to enjoy essentially normal longevity and fertility, their "gene fitness" is reduced and they will not maintain a constant gene frequency in the population from generation to generation unless new mutants replace those lost through whatever mechanism is responsible for the departure at birth from a 1:1 ratio, or unless reproductivity is increased to compensate for this loss.

SUMMARY

Congenital aniridia, or bilateral absence of the iris, is caused by an autosomal dominant gene with high penetrance and constant expression. An attempt has been made to survey all individuals with this trait living in the lower peninsula of the state of Michigan as of January 1, 1959. Through various channels, a roster of 176 aniridics distributed in 61 kindreds has been assembled. Of these, 118 cases were living in Michigan on January 1, 1959; 40 of these individuals were isolated cases and are considered to be mutants. The incidence of aniridia in Michigan is about 1.8×10^{-5}, while the mutation rate is approximately 4.0×10^{-6}/locus/generation.

Ocular examinations were conducted on 122 aniridics. The affected individual is visually handicapped, with the threat of total blindness increasing with age. Nystagmus, cataracts, and glaucoma frequently accompany the condition. The affected male is less apt to marry than the female; there is a slight decrease in male fertility.

The most striking finding to emerge from the present study is a 38:62 ratio of affected to normal children in segregating sibships instead of the expected

Mendelian 50:50 ratio. This deviation is not a function of decreased penetrance, sibship size, sex of parent, infant mortality, or heterogeneity among kindreds. Possible explanations for the deficiency of affected children are considered.

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REFERENCES

BEATTIE, P. H. 1947. A consideration of aniridia, with a pedigree. *Brit. J. Ophth.* 31: 649–676.

BELL, J. 1932. Anomalies and diseases of the eye. *Treasury of Human Inheritance*, Vol. 2, Cambridge University Press.

CROWE, F. W., SCHULL, W. J., AND NEEL, J. V. 1956. *A Clinical, Pathological, and Genetic Study of Multiple Neurofibromatosis.* Springfield, Ill.: C. C Thomas, Publisher.

DUKE-ELDER, W. S. 1941. *Textbook of Ophthalmology.* St. Louis, Mo.: C. V. Mosby Co.

FALLS, H. F. 1949. A gene producing various defects of the anterior segment of the eye. *Am. J. Ophth.* 32: 41–52.

FRANCESCHETTI, A. 1935. *Hereditary Diseases of the Eye Resulting in Blindness.* (Translated from the French by A. S. Haft.) Loaned by Army Medical Library, Washington, D.C.

FRANÇOIS, J. 1955. La gonioscopie. In *Advances in Ophthalmology.* 4: 19–129. (E. B. Streiff, ed.) Basel: S. Karger.

GLICK, P. C. 1957. *American Families.* New York: John Wiley & Sons.

GROVE, J. H., SHAW, M. W., AND BOURQUE, G. A family study of aniridia. *Arch. Ophth.* (in press).

HALDANE, J. B. S., AND SMITH, C. A. B. 1948. A simple exact test for birth-order effect. *Ann. Eugen.* 14: 117–124.

HEATH, P. 1953. Essential atrophy of the iris: a histopathologic study. *Tr. Am. Ophth. Soc.* 51: 167–192.

MANN, I. 1937. *Developmental Abnormalities of the Eyes.* Cambridge University Press.

MØLLENBACH, C. J. 1947. Congenital defects in the internal membrane of the eye. *Opera ex Domo Biologiae Heriditariae Humanae Universitatis Hafniensis.* Vol. 15. Copenhagen: Ejnar Munksgaard.

NEEL, J. V., AND SCHULL, W. J. 1954. *Human Heredity.* University of Chicago Press.

PAGANELLI, V. X. 1951. *L'aniridie bilatérale associée à la forme fruste de la maladie de Crouzon.* Thèse Genève.

PENROSE, L. S. 1955. Parental age and mutation. *Lancet* 2: 312–317.

PINCUS, M. H. 1948. Aniridia congenita. *Arch. Ophth.* 39: 60–66.

REED, T. E., AND FALLS, H. F. 1955. A pedigree of aniridia with a discussion of germinal mosaicism in man. *Am. J. Human Genet.* 7: 28–38.

REED, T. E., AND NEEL, J. V. 1959. Huntington's chorea in Michigan. 2. Selection and mutation. *Am. J. Human Genet.* 11: 107–136.

SORSBY, A. 1951. *Genetics in Ophthalmology.* London: Butterworth and Co., Ltd.

WAARDENBURG, P. J. 1932. *Das menschliche Auge und seine Erbanlagen.* The Hague: Martinus Nijhoff.

On the Incidence of Cystic Fibrosis of the Pancreas[1]

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INTRODUCTION

CYSTIC FIBROSIS of the pancreas (CF) is a hereditary disease of childhood characterized by (1) failure to gain weight despite a good appetite, (2) large abdomen, (3) fatty diarrhea, (4) liability to incur early and severe respiratory infection, (5) absence of pancreatic enzymes from duodenal fluids, (6) pancreatic fibrosis, (7) viscous mucus, and (8) elevated sodium and chlorides in sweat (see Bodian, 1952; and Shwachman, Leubner, and Catzel, 1955, for reviews). It is usually fatal before puberty and essentially no victims of the disease leave offspring. (However, see Koch *et al.*, 1960; and Marks and Anderson, 1960 for possible exceptions.) This statement is certainly true of the period prior to the introduction of antibiotics.

There is general agreement that the disease is due to a recessive gene (Andersen and Hodges, 1946; Lowe, May, and Reed, 1949; Carter, 1953; and Steinberg, Shwachman, Allen, and Dooley, 1956); but Baumann (1958) and Roberts (1960) disagree.

Baumann (1958) believes he observed too many affected sibs for a hypothesis of recessive inheritance for all CF to be correct. If we consider in detail the 42 families he examined, we find there were 63 certainly affected children and 9 probably affected out of a total of 139 children. Baumann, using single ascertainment, and accepting all 72 as affected, estimated p as .309. The correct estimate of the standard error (Baumann's value is wrong) is $\pm.047$. Hence, the estimated value does not differ significantly from .25. If any of the nine patients recorded as probably affected were not affected, the fit would be better.

Roberts concluded from a study of CF in 96 families that, "The incidence of the disease appears too high to agree satisfactorily with the quarter ratio hypothesis". He quotes Fanconi and Botsztejn (1944) as also being in disagreement; but these authors state that, "Dieses Zahlenverhältnis (the high frequency of affected sibs they observed—AGS) schliesst jedoch einem recessiven Erbgang nicht aus. . . .".

Roberts's data do not support his conclusion. Only one of the 96 families had two probands, all the others had one. Accordingly, the maximum likelihood

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method suitable for single selection (Haldane, 1938; Steinberg, 1959) is the most appropriate for analyzing his data. Accepting, as he does, all probably affected sibs as affected, the estimate of p, the proportion affected, is .300 ± .036; which is not significantly different from .25. If some of the 34 sibs listed as probably affected were not affected, the fit would be still better.

It seems reasonable to conclude that no significant proportion of CF is due to dominant or semi-dominant genes.

Two reports (Andersen and Hodges, 1946, and Goodman and Reed, 1952) indicate that the frequency of this post-natal lethal disease among the white population of the U. S. is not less than one in a thousand (.001). It is rare or absent (probably the latter) in pure Negroes (Goodman and Reed, 1952, and Steinberg, unpublished data).

The nature of the disease and its peculiar racial distribution raise the question of why the gene causing it is so frequent (~.03) among whites and absent among Negroes. Two major possibilities (not mutually exclusive) exist. One is that the mutation rate to the allele causing CF is high (~.001) in whites. The other is that the heterozygote has a selective advantage over the homozygous normal. Goodman and Reed (1952) favor the former alternative. We have hesitated to accept this interpretation because (1) it leaves unexplained the reason for the difference in the mutation rate between Negroes and whites, (2) the alternative of a selective advantage of the heterozygotes has not been investigated, and (3) methodological errors are apparent in the procedures used to estimate the frequency of the disease.

No method for detecting individual heterozygotes for CF is available. Nevertheless it is possible to estimate the fertility of these individuals if advantage is taken of the fact that at least one (and in most instances only one) paternal and one maternal grandparent of an affected child is a heterozygote. The procedure then is to determine the number of surviving offspring left by the grandparents of affected children and to compare this with the number left by an appropriate set of controls. If CF occurs with a frequency of .001, the gene frequency is approximately .031. Under these circumstances the gene can be maintained in the population if the selective disadvantage of the homozygous normal relative to the heterozygote is ~.03, i.e., if for every 100 offspring left by heterozygotes, 97 are left by homozygous normal individuals. Such a reproductive difference can be detected in a sample of a size which could be obtained, albeit with considerable expenditure of time and effort.

Before undertaking such an experiment, however, one would like to be sure that the estimate of the gene frequency is reasonably accurate. Andersen and Hodges (1946) and Goodman and Reed (1952) have estimated the frequency of CF in New York and Minnesota, respectively. We shall review their work critically.

Andersen and Hodges (1946) based their estimate on deceased children, because CF prior to 1940 was completely lethal before puberty. They determined the proportion of all *autopsied children less than 14 years of age* who died of CF in their hospital and in some other hospitals in New York City. This proved to

be three per cent. This frequency was applied to the 10,804 deaths of children under 14 years of age which occurred in New York State in 1939, to obtain 324 as the number of deaths in the state due to CF.

The calculation involves the assumptions that the patients who died with CF are a random sample of all patients coming to autopsy and that patients coming to autopsy are a random sample of all who died. If these assumptions are incorrect the estimate is incorrect. So far as we are aware the assumptions have never been tested, but experience indicates that they are unlikely to be correct.

The population base used to estimate the frequency of CF was taken as all live births in 1939 in New York State. These can have only minimal relation to the population of children (up to 14 years of age) whose deaths were used to compute the number of CF patients. Children one year old in 1939 were of necessity born before 1939. Hence there is an error in the base population of unknown magnitude. Since the deaths were for children born over a 14 year period, the base population was probably too small as an estimate of the population at risk. The sources of error enumerated above make it difficult to place reliance on Andersen and Hodges's widely quoted estimate of 1/600 as the frequency of CF.

Goodman and Reed (1952) made three estimates of the frequency of CF.

The first estimate was based on information supplied by hospital pediatricians who were asked to report the number of CF patients, and the total number of patients treated at their hospitals from January 1, 1945 through December 31, 1949. Thirty-one of 60 pediatricians who were canvassed replied. We do not know what bias this low proportion of replies introduces into the estimate. Nor do we know what bias the assumption, inherent in this procedure, that CF patients are no more likely to enter a hospital than any other child, has on the estimate.

The second estimate was based on information received from requests addressed to a 10 per cent sample of all registered pediatricians in the U. S., supplemented by replies from all pediatricians in Minnesota. The pediatricians were asked to report: The *approximate* (italics supplied) number of newborn infants they had treated since January 1, 1945 and how many of these had developed CF. Forty-six per cent of the 10 per cent sample replied to the questionnaire. As before, we do not know what bias the poor rate of reply introduces into the estimates based on these replies. Furthermore, there is no way of knowing whether the pediatricians reported only those CF patients they had first seen as newborn infants.

The third estimate was based upon a determination from death certificates of the number of deaths from CF during the years 1945–1949 among children less than 20 years of age. Finally, only deaths among children less than six years of age were accepted. This number of deaths was corrected by dividing by .42 to take into account surviving cases. The .42 was derived from the pediatricians' reports which indicated that 42 per cent of their CF patients had died at the time of their reports. The base population was taken as all live births in Minnesota during the years 1945–1949 inclusive. A major difficulty with this analysis is the

assumption that the estimate of the proportion surviving is correct. We have already alluded to the possible bias of the pediatricians' reports.

The estimates were as follows: (a) 2 per 1000, based on the hospital pediatricians' reports; (b) 1 per 1000, based on the reports of the independently practicing pediatricians; (c) 0.16 per 1000 based on deaths certainly due to CF, and 0.68 per 1000 based on deaths certainly or probably due to CF as determined from the death certificates. Goodman and Reed accept 0.7 to 1.0 per 1000 as the best estimate.

In none of the estimates made by Goodman and Reed is the base population clearly related to the CF population. For this reason and because of the various other uncertainties indicated above, we are unwilling to accept their estimates. Accordingly, we decided to obtain a new estimate of the frequency of CF based on the number of patients occurring among white children who were liveborn in Ohio during the years 1950–1953. We recognized that some CF patients would be lost through emigration but assumed that at least some of these would be compensated for by immigration.

We chose the period 1950–1953 to provide a large enough base population to make our estimate meaningful, and to have as recent a period as we conveniently could, to take advantage of the constantly improving diagnostic procedures. The cut-off point, 1953, was chosen to permit the youngest children in the study to be at least 4 years old (the study was begun in the fall of 1957) to increase the probability that mild cases would be diagnosed.

METHODOLOGY

Death certificates (not coded summaries) for all white children born during the years 1950–1953 inclusive were examined. If CF or any one of the items shown in table 1 was entered anywhere on the death certificate, it was copied. If CF as such was not recorded on the death certificate, the procedure was to obtain a transcript of the hospital record if the child had died in a hospital, or

TABLE 1. CONDENSED LIST OF ITEMS OTHER THAN CF WHICH LED TO THE COPYING OF A DEATH CERTIFICATE AND TO FURTHER STUDY OF THE PATIENT'S RECORD

Abscess of lung	Heat stroke
Atelectasis >7 days	Intestinal obstruction or adhesions due to infection
Atresia or stenosis of small intestine	Laryngotracheobronchitis
Broncheolitis, chronic	Malnutrition
Bronchiectasis	Marasmus
Bronchitis	Meconium ileus
Bronchopneumonia >4 weeks	Perforation of small intestine
Celiac disease	Peritonitis
Chronic pancreatitis	Pneumonia
Cirrhosis of liver	Prolapse of anus
Disorders of pancreatic internal secretion other than diabetes mellitus	Sprue
Enphysema, interstitial	Vitamin C deficiency
	Volvulus

of the autopsy report if a post mortem examination had been made. If such records were not available, the diagnosis on the death certificate was accepted.

The death certificates for the counties including Columbus (Franklin) and Cincinnati (Hamilton) were checked twice by two different teams. The death certificates for the county including Cleveland (Cuyahoga) were checked three times (once in the county and twice at the State Records Office in Columbus) by three different teams. Those for the remainder of the state were examined only once. The checks showed that as the team gained experience, oversights were negligible.

All pediatricians in Ohio were asked by mail, or by phone, or both, to supply us with the *names, birth dates, and, if dead, date of death* of all their patients with CF born during the years 1950–1953 inclusive. The response was 100 per cent. Similar data were requested of all major hospitals in the state, and of all hospitals in Cuyahoga County.

The Cleveland Cystic Fibrosis Foundation, which makes a determined effort to learn about all CF patients in Cuyahoga and adjacent counties, supplied us with their list of such patients.

In this study *as in all other studies of frequencies* of a disease, major sources of error are failure to diagnose a disease when it occurs and misdiagnosis of other diseases as the one in question. The former error probably occurs more often than the latter in the case of CF, but because this is the most recent study (covering the years 1950 through 1959 for diagnosis) of the frequency of the disease, errors of diagnosis are probably less important in this study than in either of the two previous studies. We have already alluded to another source of error, namely the loss of patients through emigration and the gain of patients through immigration. We do not know the magnitude of these sources of error. We assume that they are small and that they essentially balance each other and may be ignored. Finally, there is the error introduced by the failure to uncover diagnosed cases. We do not and cannot know the magnitude of this error; we have tried to minimize it.

Each of Cuyahoga, Franklin, and Hamilton Counties includes a large city with important medical centers in it. The remainder of the state is less sophisticated medically, and has fewer pediatricians per capita. We thought it more likely therefore, that in this part of the state, cases would be more often undiagnosed or, if diagnosed, treated by general practitioners and not be uncovered by us. Accordingly, the data were collected as four sets, one for each of the three counties enumerated above, and one for the remainder of the state.

The patients' addresses were reported by the Cleveland Cystic Fibrosis Foundation and they were available on the death certificates for deceased children. The addresses were not reported by the pediatricians or by the hospitals (really pediatricians located in hospitals). When the patient's address was available the patient was assigned to his usual place of residence. When the address was not available the patient was assigned to the county in which the pediatrician had his office. Since large medical centers attract patients from regions well beyond their home counties, this procedure could lead to an overestimate of

the frequency of the disease in a given county. Accordingly, attempts were made to obtain the city of residence for children reported by physicians in Cuyahoga and Franklin Counties. As a result of these efforts, the counties of residence are known for all patients reported by physicians from Cuyahoga and Franklin Counties. Ten patients were assigned to Hamilton County because they were reported by physicians resident in that county, and we did not have any addresses for the patients.

DATA

We have identified 198 CF patients in Ohio among the children born during the years 1950–1953 inclusive. Their distribution by sources of identification and by county is listed in table 2.

The distribution of the patients by the number of times they were ascertained is presented in table 3. The necessity for identifying the patients is demonstrated by these data. Thus, the 31 patients in Cuyahoga County represent 57 ascertainments (considered to be patients in the earlier studies); the 19 patients in Franklin County represent 28 ascertainments. The 198 patients represent 296 ascertainments. Eighty (40%) of the 198 were ascertained at least twice.

The incidence of CF among white children, born alive in Ohio during the years 1950–1953 inclusive, for each of the four regions and for the state as a whole is presented in tab'e 4.

Contrary to our *a priori* expectation, the incidence in the "Remaining Counties" is not lower than that in the three largest counties. In fact, it is almost identical with that in Cuyahoga and Hamilton Counties (about 26 per 100,000 or about 1 in 3800). Only Franklin County shows a higher incidence and this is

TABLE 2. NUMBER OF CF PATIENTS IDENTIFIED BY VARIOUS SOURCES IN EACH OF FOUR REGIONS IN OHIO

	Regions				
Sources[1]	Cuyahoga County	Franklin County	Hamilton County	Remaining Counties	Entire State
MD	4	12[3]	10	39[4, 5]	65
DC	0	1	7	32	40
CFF	5	0	0	14	19
MD and DC	0	5[2]	0	18	23
MD and CFF	9[2]	0	0	9[5, 6]	18
DC and CFF	11	1	0	14	26
MD, DC, and CFF	2	0	0	5[3]	7
Total	31	19	17	131	198

[1] MD = physician (pediatrician), DC = death certificate, CFF = Cystic Fibrosis Foundation.

[2] Two were each reported by two physicians.

[3] One was reported by two physicians.

[4] Four were reported by two physicians.

[5] One was reported by three physicians.

[6] Three were reported by two physicians.

TABLE 3. THE DISTRIBUTION OF PATIENTS BY THE NUMBER OF TIMES THEY WERE ASCERTAINED

No. of Ascertainments	Region									
	Cuyahoga County		Franklin County		Hamilton County		Remaining Counties		Entire State	
	No.	%	No.	%	No.	%	No.	%	No.	%
1	9	29.0	12	63.2	17	100.0	80	61.1	118	59.6
2	18	58.1	5	26.3	0	—	41	31.3	64	32.3
3	4	12.9	2	10.5	0	—	8	6.1	14	7.1
4	0	—	0	—	0	—	2	1.5	2	1.0
Total Patients	31	100.0	19	100.0	17	100.0	131	100.0	198	100.0
Ascertainments	57		28		17		194		296	

TABLE 4. INCIDENCE OF CF PATIENTS AMONG WHITE CHILDREN BORN ALIVE IN OHIO DURING THE YEARS 1950–1953 INCLUSIVE

	Location				
	Cuyahoga County	Franklin County	Hamilton County	Remaining Counties	Entire State
Live Births	122,090	49,296	65,940	504,837	742,163
CF Patients	31	19	17	131	198
Incidence of CF:					
1 in	3,938	2,595	3,879	3,854	3,748
as decimal	.000254	.000385	.000258	.000260	.000267
95% confidence intervals:					
Upper	.000307	.000494	.000335	.000284	.000287
Lower	.000191	.000254	.000163	.000233	.000245

not significantly different from the incidence found in the other regions ($\chi^2_3 = 2.880$, $.5 > P > .3$). In the absence of evidence to the contrary, we have pooled the data for the four regions and have arrived at an estimate of 27 per 100,000 with a 95% confidence interval of 29 to 25. (These rates are approximately equivalent to 1 in 3700 with a 95% confidence interval of 1 in 3500 to 1 in 4000.)

DISCUSSION

Our estimate of the incidence of CF (1 in 3700) is considerably lower than Andersen and Hodge's (1946) estimate of 1 in 600, and Goodman and Reed's (1952) estimate of 1 in 1000. We have already described the sources of error in their procedures which we believe make their estimates too high. Our estimate may be too low because of the undiagnosed cases we could not detect and because of the diagnosed cases we may have failed to detect. It seems unlikely, although we cannot prove it, that we have detected only about one-quarter of the cases in Ohio. We may be reasonably certain, therefore, that the incidence of CF in Ohio is not 1 per 1000.

The improved methods of diagnosis (not available at the time of the earlier studies), the widespread interest in CF (stimulated by the improved methods of diagnosis, and of treatment, and by the activity of various CF foundations), combined with the complete response of the hospitals and of the pediatricians, lead us to believe that our estimate is probably of the right order of magnitude.

If we accept the estimate of 0.000267 as the incidence of CF; the estimate of the frequency of the recessive gene leading to the disease is ~.016. A lethal gene occurring with this frequency may be maintained in a population in equilibrium, in the absence of mutation, with a heterozygous advantage of approximately 1.6 per cent. It is impractical to attempt to measure such an advantage in a society such as ours.

The present estimate of the incidence of CF, 1 in 3700, while considerably lower than previous estimates, still leaves CF as the most frequent lethal genetic disease among white children. We are still left with the problem of accounting for this high incidence among the white population and the essentially zero incidence among the Negro population. A reasonable *a priori* explanation is heterozygote advantage combined with a low mutation rate. The required advantage, as we have seen, is probably too small to be measured, in terms of increased fecundity, in our society. An alternative approach is to seek a clinical rationale for this advantage. We leave this problem to the clinicians.

The incidence of 1 in 3700 for CF patients means that about three per cent of the population is heterozygous for the recessive gene leading to CF. Hence, the risk that a healthy sib of a CF patient may marry a heterozygote assuming no consanguinity, is .03.

SUMMARY

The incidence of cystic fibrosis among white children born alive in Ohio during the years 1950–1953 inclusive, was determined by a search of death certificates, correspondence and telephone conversations with all pediatricians in Ohio, and with the aid of the Cleveland Cystic Fibrosis Foundation. The incidence, 198/742, 163 or .000267 (1/3700), is about one quarter that of previous estimates.

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REFERENCES

ANDERSEN, DOROTHY H. AND R. G. HODGES. 1946. Celiac syndrome V. Genetics of cystic fibrosis of the pancreas with a consideration of the etiology. *Am. J. Dis. Child.* 72: 62–80.

BAUMANN, T. 1958. Die Mucoviscidosis als recessives und irregulär dominantes Erbleiden. *Helvetica paediat. acta* 13: supp. 8.

BODIAN, M. 1953. *Fibrocystic disease of the pancreas.* New York: Grune and Stratton.

CARTER, C. O. 1953. in M. Bodian. *Fibrocystic disease of the pancreas.* New York: Grune and Stratton.

FANCONI, G. AND A. BOTSZTEJN. 1944. Die Familiäre Pankreasfibrose mit Bronchiektasien. *Schweitz. Med. Wsch.* 74: 85–93.

GOODMAN, H. O. AND S. C. REED. 1952. Heredity of fibrosis of the pancreas. Possible mutation rate of the gene. *Am. J. Human Genet.* 4: 59–71.

HALDANE, J. B. S. 1938. The estimation of the frequencies of recessive conditions in man. *Ann. Eugen.* 8: 255–262.

KOCH, E., H. BOHN, W. RICK, UND W. HARTUNG. 1960. Der erbliche Mucoviscidosis des Erwachsenen als unerwartet häufige Ursache chronischer Bronchialleiden und ihrer Folgen. *Der Internist* 1: 35–44.

LOWE, C. U., C. D. MAY, AND S. C. REED. 1949. Fibrosis of the pancreas in infants and children. *Am. J. Dis. Child.* 78: 349–374.

MARKS, B. L. AND C. M. ANDERSON. 1960. Fibrocystic disease of the pancreas in a man aged 46. *Lancet* i: 365–367.

ROBERTS, G. B. S. 1960. Familial incidence of fibrocystic disease of the pancreas. *Ann. Human Genet.* 24: 127–135.

SHWACHMAN, H., H. LEUBNER, AND P. CATZEL. 1955. Muscoviscidosis. in *Advances in Pediatrics.* Vol. 7. New York: Year Book Publishers.

STEINBERG, A. G. 1959. Methodology in human genetics. *J. Med. Ed.* 34: 315–334.

STEINBERG, A. G., H. SHWACHMAN, F. H. ALLEN, JR., AND R. R. DOOLEY. 1956. Linkage studies with cystic fibrosis of the pancreas. *Am. J. Human Genet.* 8: 162–176.

Family Limitation Based on Family Composition

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INTRODUCTION

AMONG POPULATION GROUPS where birth control is widely practiced, the decision to limit family size may be based on the family composition as it is sequentially obtained during the course of reproductive history. We may envision two types of circumstances where such selective limitation may arise. It is well known that a preference for children of one sex or a desire for children of both sexes may lead parents to limit the size of their sibship after a favorable sex combination has been obtained. Data collected by Dahlberg (1948), Myers (1949) and Fancher (1956) in Sweden and America suggest that parents who have a mixture of sexes among their offspring are less likely to continue procreation than those with offspring all of one sex. From a somewhat different viewpoint, parents may be discouraged from continuing child bearing after the birth of a child with some defect they consider to be hereditary or likely to appear in a later birth. If the belief precedes the birth of the first affected child, parents may decline the risk of producing a second, in which case, the termination rule may be regarded as the first occurrence. Without this preconception, the birth of a second affected child may lead them to the same decision, in which case, the termination criterion is the second occurrence. It is the present intention to examine the effect of such limitation practices on the composition of sibships otherwise selected in a random fashion.

Since parents at present cannot exercise control over gamete formation or select particular gametes to participate in fertilization, intuition might lead one to the conclusion that selective limitation based on sibship composition should produce an effect on the birth order sequence of the characteristic under study but not on the expected proportion of individuals with the characteristic. This point may be explored by examining the expected proportion of males or affected individuals in a group of sibships where the termination criterion is based on composition in some specified manner.

GENERAL MODEL

Each couple at the outset of reproduction may be thought of as possessing a "potential" family size N jointly determined by the interplay of fecundity and by the desire for no more than a given number of children, say "maximum allowable" size. Since couples differ in their ability to reproduce as well as in their

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concept of maximum, N will vary between couples. The actual number of children produced during the course of the reproductive history of a given couple, say realized size T, will depend on their termination rule and the value of their potential size, N being the upper limit for T. The maximum allowable size may be exceeded because of the parents' inability to prevent conception or may not be attained because of their relative infertility. In addition, the realized size T may fall short of N because a particular composition meeting the termination criterion is attained prior to the N*th* birth. Two types of stoppage rules will be considered—termination with the first r occurrences, which applies both to sex preference and to the fear of producing additional affected children, and termination with the first occurrence of one of each type of outcome prior to the N*th* birth, which furnishes a crude analogy with the desire for representation of both sexes in a sibship.

In order to formalize these considerations, we shall make use of the following random variables and corresponding frequency functions:

N—the potential sibship size or the composition-independent limit on size.

T—the realized size.

M—the number of individuals with the characteristic in the sibship. (1)

$f_n = \Pr(N = n)$ the frequency function of N.

$g_{jn} = \Pr(M = j \mid N = n)$ the conditional frequency function of M.

$h_{kn} = \Pr(T = k \mid N = n)$ " " " " " T.

Under this setup, we shall assume the unconditional probabilities of M and T to be given by the terms of the compound distributions

$$\Pr(M = j) = \sum_n f_n \cdot g_{jn} \qquad \Pr(T = k) = \sum_n f_n \cdot h_{kn} \tag{2}$$

which implies that the form of the conditional probabilities is the same for all values of n.

M, the number of individuals with the characteristic in a sibship, will be taken as the sum of k independent and identically distributed binomial variables, i.e.:

$$M = X_1 + X_2 + \cdots + X_k$$

$$\text{where } X_\alpha = \begin{cases} 0 \text{ if } \alpha th \text{ child hasn't the characteristic} \\ 1 \text{ if } \alpha th \text{ child has the characteristic} \end{cases}$$

$$\Pr\left\{X_\alpha = \begin{matrix}1\\0\end{matrix}\right\} = \begin{cases} p \\ q \end{cases}$$

The underlying assumption is that the probability a sib has the characteristic is constant and equal between and within sibships. This roughly applies when the character is maleness or some simple recessive trait uncomplicated by incomplete penetrance.

The appropriate form for f_n, the frequency function of N, will depend on the fertility and family limitation practices of the population under study. Lotka (1939) found certain American family statistics to be described by the terms of a geometric series. Kiser and Whelpton (1944), in their Indianapolis fertility study, display size distributions which are adequately fitted by Poisson series. For purposes of illustration, both forms will be applied in the present context.

TERMINATION WITH THE FIRST OCCURRENCE

Limitation of family size at the birth of the first r males or the first r affected children prior to the *nth* birth will provide an analogy with parental birth control practice as it appears to exist in certain population groups. In the simplest case, we may consider couples who terminate at the birth of the first male child prior to the *nth* birth. Under this rule, parents will stop at the first birth if male, at the second if the first is female and second male, and so on up to the *nth* birth in which case they will stop regardless of the sex either because of inability to continue or because n is the maximum allowable size. The probabilities of the various possible sex combinations for parents with upper limit n are seen to be

$$\Pr(M = 0, T = n \mid N = n) = q^n$$

$$\Pr(M = 1, T = k \mid N = n) = pq^{k-1}$$

From (1), the conditional distribution of M is given by

$$g_{0n} = \Pr(M = 0 \mid N = n) = q^n$$

$$g_{1n} = \sum_{k=1}^{n} pq^{k-1} = 1 - q^n$$

For N a geometric variable as in Lotka's data, we may let $f_n = (1 - a)a^{n-1}$, a series truncated at the "0" class which implies that size 0 families are not observable. The single parameter a has range $0 < a < 1$, typical values falling between .7 and .9. From (2), the unconditional distribution of M becomes:

$$\Pr(M = 0) = \sum_n f_n \cdot g_{0n} = \frac{(1 - a)q}{1 - aq}$$

$$\Pr(M = 1) = \sum_n f_n \cdot g_{1n} = \frac{p}{1 - aq}$$

Similarly, for realized size T

$$\Pr(T = k \mid N = n) = h_{kn} = \begin{cases} pq^{k-1} & \text{for } k < n \\ pq^{n-1} + q^n & k = n \end{cases}$$

The unconditional distribution of T is obtained by compounding h_{kn} with f_n which turns out to be the geometric series

$$\Pr(T = k) = \sum_n f_n \cdot h_{kn} = (1 - aq)(aq)^{k-1} \quad \text{for } k > 0$$

The joint probability of M and T is given by

$$\Pr(M = 0, T = k) = (1 - a)q(aq)^{k-1}$$
$$\Pr(M = 1, T = k) = p(aq)^{k-1}$$

The moments of M and T may be obtained directly from the unconditional distributions and have expressions

$$E(T) = \frac{1}{1 - aq} \qquad E(M) = \frac{p}{1 - aq} = p \cdot E(T)$$
$$V(T) = \frac{aq}{(1 - aq)^2} \qquad \mathrm{Cov}(MT) = 0 \tag{3}$$

Hogben (1952), who has investigated a similar situation involving stoppage at the birth of the first, second, and so forth up to the *rth* child with some congenital defect for fixed potential size n, concluded that selective termination of this type produces no effect on the expected proportion of affected children because the ratio of the expectations of M and T is p, i.e.

$$\frac{E(M \mid N = n)}{E(T \mid N = n)} = p$$

Thus, he reasoned that the usual estimator of p, the ratio $R = M/T$, is unbiassed. Since the ratio of the expectations of two random variables is not generally equal to the expectation of the ratio, the issue may be settled by examining the expectation of the estimator R since, by definition, R will be unbiassed if its expectation is p. R has frequency function

$$\Pr(R = 0) = \frac{(1 - a)q}{1 - aq} \qquad \text{and} \qquad \Pr\left(R = \frac{1}{k}\right) = p(aq)^{k-1}$$

The expected value of R is seen not to be p but

$$\begin{aligned} E(R) &= \frac{1}{k} \cdot \Pr\left(R = \frac{1}{k}\right) \\ &= p\left(1 + \frac{aq}{2} + \frac{(aq)^2}{3} + \frac{(aq)^3}{4} + \cdots\right) \\ &= \frac{p}{aq}\left(-\log_e(1 - aq)\right). \end{aligned}$$

The ratio estimator R has a positive bias B which in general cannot be disregarded.

For w sibships operating under the rule of terminating at the first birth of a male or affected child, in analogy with the maximum likelihood estimator of p under the binomial assumptions of constant p and fixed n, the usual estimate of p is taken to be the statistic

$$r_w = \sum m_i / \sum t_i$$

where m_i and t_i are the number of males and the total number of children in the *ith* sibship. The expectation of the corresponding random variable R_w may be approximated by the following formula due to Sukhatme (1958):

$$E(R_w) \sim \frac{E(M)}{E(T)}\left[1 + \frac{1}{w}\left\{\frac{V(T)}{E^2(T)} - \frac{Cov(MT)}{E(T)E(M)}\right\}\right]. \tag{4}$$

Under the present geometric assumption for f_n, substituting the expressions for the moments of M and T from (3), the approximate bias is given by

$$B_w \sim \frac{apq}{w} \tag{5}$$

As the number of families w increases, B_w decreases and R_w is thus a consistent estimator of p.

In certain populations, it may be more appropriate to treat potential sibship size N as a Poisson variable with parameter θ, in which case the joint probability of M and T is given by

$$\begin{aligned} Pr(M = 0,T = k) &= e^{-\theta}\frac{\theta^k}{k!}\cdot q^k \\ Pr(M = 1,T = k) &\sum_{n=k}^{\infty} e^{-\theta}\frac{\theta^n}{n!}\cdot pq^{k-1}. \end{aligned} \tag{6}$$

These expressions are related to the fact that, under stoppage rule r = 1, parents will continue reproduction until either M = 1 or T = n, whichever occurs first. Thus, parents with no males will continue until the family size has attained the maximum value n with probability f_n. The probability of obtaining exactly one male among k children will be given by

$$\sum_{n=k}^{\infty} f_n \cdot pq^{k-1}$$

since the decision to stop is made at the birth of the first male, an event which may occur at the *kth*, (k + 1)*th*, $\cdots$ birth.

Under the Poisson assumption for f_n, the first two moments of M and T turn out to be

$$\begin{aligned} E(T) &= \frac{1}{p}[1 - e^{-\theta p}] \qquad E[T(T-1)] = \frac{2q}{p^2}[1 - e^{-\theta p}(1 + \theta p)] \\ E(M) &= [1 - e^{-\theta p}] \qquad E(MT) = \frac{1}{p}[1 - e^{-\theta p}(1 + \theta p)]. \end{aligned} \tag{7}$$

Substituting the formulae for the moments of M and T in (4), we obtain for the expectation of the ratio estimator of p for f_n Poisson

$$E(R_w) \sim p\left[1 + \frac{q}{w}\left\{\frac{1 - e^{-\theta p}(1 + \theta pq)}{(1 - e^{-\theta p})^2}\right\}\right].$$

As in the geometric case, the ratio estimator of p is seen to be positively biassed and consistent.

The decision to terminate reproduction at the birth of the first male or first affected child is seen to produce an effect on the expected proportion of males or affected children in a random selection of fraternities. For investigation of the sex ratio, the bias will be of negligible magnitude because, typically, such studies involve large numbers of families. Difficulties are more likely to be encountered in genetic studies where w may tend to be small.

TERMINATION WITH THE FIRST R OCCURRENCES

In more general terms, when the decision to terminate is made at the birth of the first r boys or the first r affected children, the probability a sibship has j males among k total children is

$$\Pr(M = j, T = k) = \begin{cases} f_k \cdot \binom{k}{j} p^j q^{k-j} & \text{for } j < r \\ \sum_{n=k}^{\infty} f_n \cdot \binom{k-1}{r-1} p^r q^{k-r} & j = r \end{cases} \tag{8}$$

This expression is the consequence of the stoppage rule r which imposes the condition that parents with fewer than r males will continue until the total size attains the upper limit $N = k$ with probability f_k. Since stoppage is at the first r males, parents obtaining exactly r males will consist of those with upper limits $N = k, k + 1, k + 2, \cdots$

We may proceed in the same manner as with stoppage at the first male ($r = 1$) and obtain expressions for the moments of M and T assuming a particular functional form for f_n. For N geometric as before, $f_n = (1 - a)a^{n-1}$, the expectations of M and T may be shown to be equal to

$$E(T) = \frac{1 - z^r}{1 - a} \qquad \text{where} \quad z = \frac{ap}{1 - aq}$$

$$E(M) = p \cdot E(T)$$

Expressions for the variance of T and the covariance of M and T do not reduce to a convenient algebraic form and will be left unstated. The bias in the ratio estimator of p may again be approximated by (4) which reduces to

$$B_w \sim \frac{apq}{w} rz^{r-1} \frac{(1 - z)^2}{(1 - z^r)^2}$$

Whatever distributional form is assumed for f_n, the bias will generally not be zero. Since the number of families appears in the denominator of B_w, the estimator R_w is consistent in that B_w tends to 0 as w becomes large.

The implication of this result from the standpoint of the geneticist is apparent. In drawing inferences regarding the transmission genetics of a given trait, human geneticists utilize estimates of p based on sibships which may be obtained

by the sequential decision process under consideration. The usual estimate of p, which turns out to be the maximum likelihood estimate when ascertainment is by sibships, will be positively biassed if the parents practice selective limitation. If the bias is sufficiently great, the inference on the mechanism of inheritance may be incorrect. Since the estimator of p is consistent or asymptotically unbiassed, this suggests that the problem may be minimized by taking larger samples of families.

DESIRE FOR BALANCE

Empirical data collected in Western countries suggest that parents are more likely to continue bearing children when their prior offspring are all of one sex than when a mixture of the sexes has been obtained. For parents behaving in this manner, we may assume continuance up to $N = n$ children unless at least one of each sex has been obtained. Thus, parents for whom $n = 3$, will either stop at two children (one male, one female) with probability 2pq or stop at three with probability $1 - 2pq$. Similarly, parents with $n = 4$ will stop at two, three or four children with probabilities 2pq, pq and $p^3 + q^3$ respectively, and so on.

The crudeness of this analogy with what occurs in practice is recognized. The concept of a fixed maximum size at the outset of reproduction takes no cognizance of learning through experience. A maximum size of four may be directly downgraded to two children as a result of experiencing the first two whatsoever be their sexes. A variety of factors may further confuse the picture—loss of children, divorce or death of a parent, and remarriage. The fact remains, that among completed two child families listed in Who's Who in America (see Fancher, 1956), there is a striking excess of the male-female sets. The most economical explanation for this excess is to be found in the termination of reproduction when both sexes are represented in the sibship.

Under stoppage at the first occurrence of both a male and a female in the sibship, the joint probability of M, the number of males, and T, the total size, is given by

$$\begin{aligned} \Pr(M = 0, T = k) &= f_k \cdot q^k & k &= 1, 2, \cdots \\ \Pr(M = 1, T = k) &= \sum_{n=k}^{\infty} f_n \cdot pq^{k-1} & k &= 2, 3, \cdots \\ \Pr(M = k - 1, T = k) &= \sum_{n=k}^{\infty} f_n \cdot qp^{k-1} & k &= 2, 3, \cdots \\ \Pr(M = k, T = k) &= f_k \cdot p^k & k &= 1, 2. \cdots \end{aligned} \tag{9}$$

These probabilities are obtained through consideration of the termination rule, the first occurrence of a male and a female in the sibship. Parents obtaining children of only one sex will continue until their upper limit on sibship size has been obtained. Parents with upper limits k, $k + 1$, $k + 2$, etc., will terminate at the *kth* birth if the *kth* represents the first occurrence of a male and a female in the sequence.

For N a geometric variable with $f_n = (1 - a)a^{n-1}$, the moments of M and T may be shown to be equal to

$$E(T) = \frac{1 - a^2pq}{(1 - ap)(1 - aq)} \qquad E(M) = p \cdot E(T)$$

$$E(T^2) = \frac{1 + ap}{(1 - ap)^2} + \frac{1 + aq}{(1 - aq)^2} - 1$$

$$E(MT) = p\left[\frac{1}{(1 - aq)^2} + \frac{1 - a}{(1 - ap)^2} - 1\right].$$

For $w = 1$, the variable $R = M/T$ has expectation

$$E(R) = p + a^2pq(q - p)/3 + a^3pq(q^2 - p^2)/4 + \cdots$$

When $p = q = \frac{1}{2}$, the bias of the ratio estimator is seen to be 0 as might be anticipated. The bias is positive when $p < \frac{1}{2}$ and negative when $p > \frac{1}{2}$.

For w sibships whose parents terminate according to the "at least one of each" rule, the ratio estimator of p has approximate bias

$$B_w \sim \frac{apq}{w \cdot E^2(T)}\left[\frac{1}{(1 - aq)^2} - \frac{1}{(1 - ap)^2}\right] \tag{10}$$

Examination of (10) shows that $B_w = 0$ when $p = q$, is positive when $p < q$ and negative when $p > q$, in accord with the exact result obtained above for $w = 1$. Similar but algebraically more complex results are obtained when f_n is assumed to be a Poisson series.

SUMMARY AND CONCLUSIONS

a. A general model has been formulated for analyzing the distribution of qualitative characters in sibships when sibship size is dependent on the composition as it is sequentially obtained during the course of reproductive history. Such a situation arises in practice when parents limit their families on the basis of producing a child or children with a given defect or after obtaining a desired sex combination among their offspring.

b. The model begins with the definition of "potential" family size determined by fecundity and by the desire for no more than a given number of children and with the assumption of constancy for the probability, p, a child has the characteristic under study. General expressions are derived for the probability a sibship is of a particular composition under two types of termination rules.

c. Under termination at the birth of the first r children with the characteristic, specific formulae are derived for the two cases when "potential" size is described by a geometric series and by a Poisson series. It is demonstrated that the usual estimator of p is positively biassed but consistent. This result implies that small samples of families selectively terminated will lead to an overestimate of the segregation ratio in genetic studies. Since the estimator is consistent, the bias will be reduced by increasing the number of families in the sample.

d. When parents terminate with the production of at least one boy and one girl in their sibship, a general expression for the probability of the various possible family sex combinations is obtained. The estimator of p is shown to be biassed except when $p = \frac{1}{2}$.

REFERENCES

Bernstein, M. E. 1952. Studies in the human sex ratio. 2. The proportion of unisexual sibships. *Human Biol.* **24**: 35–43.

Dahlberg, G. 1948. Do parents want boys or girls? *Acta Genet. et Stat. Med.* **1**: 163–167.

Fancher, H. L. 1956. The relationship between the occupational status of individuals and the sex ratio of their offspring. *Human Biol.* **28**: 316–322.

Hogben, L. 1952. Selective limitation of sibship size. *Brit. J. Social M.* **6**: 188–189.

Kiser, C. V. and Whelpton, P. K. 1944. Variations in the size of completed families of 6,551 native white couples in Indianapolis. *Milbank Mem. Fund Q.* **22**: 72–105.

Lotka, A. 1939. *Theorie analytique des associations biologiques. Vol. II. Actualites scientifiques et industrielles, No. 780.* Paris: Hermann et Cie.

Myers, R. J. 1949. Same sexed families. *J. Hered.* **40**: 268–270.

Sukhatme, P. V. 1958. *Sampling theory of surveys with applications.* Iowa State College Press.

Inheritance of Primary Systemic Amyloidosis

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AMYLOIDOSIS is characterized by the deposition within tissues of a complex material called amyloid. This term was proposed by Virchow (1860) on the basis that this substance reacted with iodine and sulfuric acid in a manner comparable to starch.

Primary systemic amyloidosis is a complex entity exhibiting a protean range of clinical signs and symptoms. It occurs without any obvious predisposing factors, as compared to *secondary* amyloidosis which is associated with chronic disease states such as myelomatosis, rheumatoid arthritis, tuberculosis or other chronic infections. Once considered to be a rare pathological condition, primary amyloidosis appears to be increasingly more common, or at least to be diagnosed more frequently, as evidenced by numerous recent reports of the disease. Although the exact etiology of this disease is unknown, some studies suggest important hereditary factors as a cause of this condition for which there is no known effective treatment.

The report by Maxwell and Kimbell (1936) of primary systemic amyloidosis in three brothers was the earliest implication of genetic factors in this entity. De Navasquez and Treble (1938) found amyloid deposits in the peripheral nervous system of a 52 year old carpenter whose sister and two brothers had died of a clinically similar condition. Ostertag (1950) reported amyloidosis in two brothers with suggestive evidence that their mother, a sister, and an offspring of one of the brothers had had the same disease. Isaak (1950) indicated an association of amyloidosis involving the skin with psoriasis in 2 siblings. Andrade (1952) described a peculiar form of peripheral neuropathy associated with amyloidosis. This occurred as an "endemic" disease in a Portuguese fishing area with 51 of 64 cases occurring in twelve families. The pedigrees of two of the families observed by Andrade were presented by Horta (1956) (see Fig. 1). Kantarjian and DeJong (1953) observed primary amyloidosis in two sisters and their father. Shulman and Bartter (1956) reported histologically established amyloidosis in a mother, her son and daughter by different fathers. A more detailed study of this family with special reference to the eye findings was pub-

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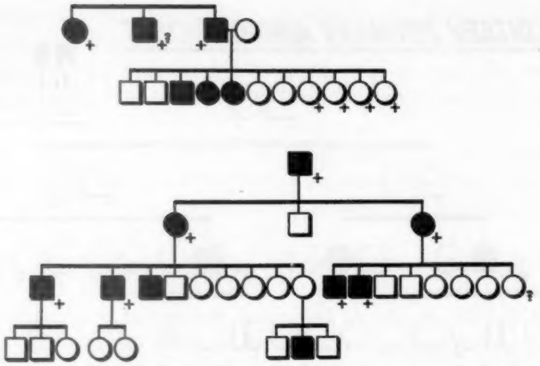

FIG. 1. Pedigree of the distribution of clinical evidence of amyloidosis in two of Andrade's Portuguese families as reported by Horta (1956). Affected individuals are indicated by solid squares or circles. The crosses indicate that these individuals are deceased.

lished by Kaufman in 1958. Kaufman and Thomas (1959) discussed the vitreous opacities present in primary amyloidosis and added another family having 2 brothers affected. They described an additional family of a 62 year old man with vitreous opacities and a positive gingival biopsy for amyloidosis. Three younger brothers had symptoms and signs suggestive of primary amyloidosis although the disease had not definitely been established. A review of these three families (seen at the National Institutes of Health) was presented in a clinical staff conference report with von Sallman (1960) as moderator. Van Allen (1960) noted primary amyloidosis in four brothers of a family of eleven siblings. The disease was also established in a son of one of the definitely affected brothers and in the son of a brother suspected but not proven to have the disease. It was likely that the disease was also present in another sibling and two other members of the second generation of this family. These studies suggest that primary amyloidosis may be inherited as an autosomal dominant trait. The true importance of hereditary factors in this disease will only be ascertained eventually by a thorough investigation of the families of a large series of cases.

Amyloidosis has also been reported by Tuqan (1958) and Mamou (1955) in association with "periodic disease". The latter condition has been suggested by Reimann et al. (1954) to be inherited as a dominant trait. The significance of the association of amyloidosis with periodic disease (also termed paroxysmal peritonitis or periodic fever) remains to be established. Heller, Sohar, and Sherf (1958), however, apparently believed that the amyloidosis was secondary to the periodic disease rather than a primary condition.

The purpose of this investigation is to present further evidence of an hereditary factor in primary amyloidosis. This study was initiated when two first cousins (indicated by arrows in Fig. 2 which shows a small portion of the pedigree of this large family) were seen at the University of Michigan Medical Center exhibiting ocular changes similar to those previously noted in the two sisters with

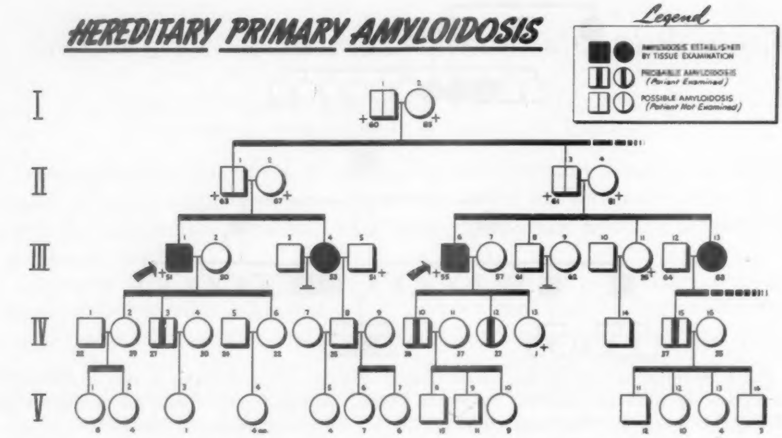

FIG. 2. The pedigree of a portion of the family reported in this investigation.

amyloidosis reported by Kantarjian and DeJong (1953). A positive biopsy established the diagnosis of primary amyloidosis in each of these cousins. Subsequently genetic and biochemical studies of this family were reported by Rukavina, Block, Jackson, Falls, Carey and Curtis (1956).

GENETIC STUDIES

Fig. 2 shows the propositus of the study, III-1, who was originally seen because of a progressive visual acuity loss secondary to extensive sheet-like vitreous opacities. A disabling peripheral neuropathy was also present. Death occurred suddenly at the age of 51; necropsy findings substantiated the clinical diagnosis in that widespread amyloid deposition was found in the heart, tongue and in the walls of the blood vessels of almost all organs. His 27 year old son, IV-3, showed changes about the vessels of the eye suggestive of amyloidosis as described by Falls, Jackson, Carey, Rukavina, and Block (1955). A sister, III-4, had symptoms of congestive heart failure and a peripheral neuropathy. The clinical diagnosis was established by a positive intestinal mucosal biopsy although several earlier biopsies were negative. (This information was kindly furnished by Dr. Sam Fox, III, and Dr. Leonard Laster of the National Institutes of Health, Bethesda, Maryland.)

III-6 (Fig. 2), a first cousin of III-1, also had progressive visual loss, a peripheral neuropathy and manifestations of cardiac failure. Biopsy revealed amyloid infiltration of the systemic blood vessels. This individual died suddenly at age 55 and post mortem examination disclosed extensive perivascular and cardiac amyloid deposition. The vitreous opacities were established by histological examination as representing amyloid deposition, as has also been reported by Kaufman (1958) and by Kaufman and Thomas (1959). III-6's sister, III-13,

had a disabling peripheral neuropathy and vitreous opacities. A skin biopsy was positive for amyloid. Several of her children presented clinical findings suggestive of primary amyloidosis. III-6's father, II-3, was not examined but apparently had a peripheral neuropathy, loss of eyesight, and died of congestive heart failure.

Bone marrow and x-ray studies of the individuals in these families have revealed no evidence of multiple myeloma, a disease with which amyloidosis has been found to be associated and which may possibly be hereditary as suggested by Nadeau, Magalini and Stefanini (1956).

I-1 and I-2 (Fig. 2) came to the United States with their family from Switzerland in 1883. One hundred fifty-six of the two hundred fifty-two descendants of this couple have been examined and a positive tissue diagnosis of primary amyloidosis has been established in four members of the pedigree (Fig. 2). Suggestive signs and symptoms of primary amyloidosis were present in many other individuals of this family. Information on the ancestors of I-1 and I-2 has been obtained from Swiss Community records as far back as 1733 and has *failed to reveal any evidence of consanguinity* within this particular family. With the information that the two brothers, II-1 and II-3 (fathers of the propositi) were affected, it would seem likely that the disease was inherited from father to son—probably excluding sex linkage. The report of Van Allen (1960) of an affected father and son would also tend to eliminate a sex linked dominant type of inheritance. A review of the pedigree of this family would suggest that the mode of inheritance is autosomal dominant. This agrees with the impression obtained by a review of the other reported families of primary amyloidosis.

Amyloidosis has been known to be a disease of variable manifestation and severity. Even within the reported family this interesting variability of expressivity was noted. However, the presence of a peripheral neuropathy, with onset in the third and fourth decades, has been a consistent clinical feature. In the Portuguese families reported by Andrade (1952) and Horta (1956), frequent peripheral nerve involvement of the lower extremities contrasts with the upper extremity involvement noted in this study. In their cases the disease became detectable also in the third and fourth decades. Death followed onset of symptoms within ten years in their series, which is much earlier than has been noted in this family. Thyroid involvement seems to be a frequent occurrence in hereditary primary amyloidosis as has been observed in the cases reported by Van Allen, Kantarjian and DeJong, and Shulman and Bartter, as well as in this family. Kaufman and Thomas (1959) have emphasized that the presence of vitreous opacities may serve as an important adjunct in separating the familial from the sporadic forms of primary amyloidosis. They also mentioned the infrequency of macroglossia in the familial form of the disease. This has been noted in this family although III-4 (Fig. 2) showed only a moderately enlarged tongue, and III-1 (Fig. 2) showed marked amyloid infiltration in the tongue, but it was not enlarged clinically. Although this entity exhibits considerable variability of expression within the kindred, a surprising similarity of clinical manifestations does exist within the family.

BIOCHEMICAL STUDIES

Since amyloidosis is a disease with such variable manifestation the diagnosis can not be *established* clinically. The only dependable definitive diagnostic procedure in individuals suspected of having the disease is microscopic examination of tissues obtained by biopsy or at necropsy. Even tissue examination may require special staining techniques at times. In view of this, biochemical studies were initiated in this family with the hope of detecting some biochemical aberration which would be of definitive aid in establishing the diagnosis of primary amyloidosis.

Studies of serum proteins by free, moving boundary, electrophoresis revealed an unusual peak in the α_2 globulin area in the serum of the two cousins seen initially as reported by Block, Rukavina, and Curtis (1955). Subsequent investigations revealed a similar abnormality in 29 of 66 individuals of this family as reported by Block, Rukavina and Curtis (1956). Some correlation of this atypical serum protein pattern was found with the clinical evidence of disease within this family. Electrophoretic studies of the individuals of ths family have continued in an effort to define more clearly the relationship of this serum protein change to the entity. The finding of a similar α_2 globulin abnormality in two individuals of this family who did not show amyloidosis at autopsy has suggested that the α_2 globulin change may be non-specific. No α_2 globulin abnormality was noted in a suspected case of primary amyloidosis seen by H.F.F. although a positive diagnosis was later established and reported by Kaufman and Thomas (1959). Certainly further electrophoretic studies are indicated in patients with primary amyloidosis. No consistent abnormality in affected individuals of this family was detected by the starch gel technique of Smithies (1955) or by immunoelectrophoresis in seven members as performed by Riva (1958). Rukavina, Block and Curtis (1956) reported lipoprotein aberrations in affected members of this family which deserve further investigations. We have been unable to show any consistent abnormalities by paper electrophoretic analyses for lipoproteins or glycoproteins.

A study of the serum hexosamine level of 11 individuals of this family has revealed no alteration from normal as reported by Jackson, Block and Ratliff (1960). Calkins and Cohen (1959) reported an elevation of serum hexosamine in eight of nine patients with primary amyloidosis with the normal value being present in a patient with the "familial variety of the disease". This report and the normal findings in affected individuals of this family would suggest that this determination might be of value in differentiating hereditary from nonhereditary primary amyloidosis. This finding would also seem to corroborate the clinical impression presented by Kaufman and Thomas (1959) that the hereditary disease differs from that seen in sporadic cases of primary amyloidosis.

SUMMARY

A genetic study of primary systemic amyloidosis is presented as manifested within a large family group. Evidence from this family suggests that the trait is inherited as an autosomal dominant condition. This is in agreement with the impression suggested by the other reported families with this disease.

REFERENCES

ANDRADE, C. 1952. A peculiar form of peripheral neuropathy; familial atypical generalized amyloidosis with special involvement of peripheral nerves. *Brain* **75**: 408–427.

BLOCK, W. D., RUKAVINA, J. G., AND CURTIS, A. C. 1955. An atypical electrophoretic peak in serum of patients with familial primary systemic amyloidosis. *Proc. Soc. Exp. Biol.* 89: 175–177.

BLOCK, W. D., RUKAVINA, J. G., AND CURTIS, A. C. 1956. Serum electrophoretic studies on patients with familial primary systemic amyloidosis. *J. Laborat. Clin. M.* 47: 357–364.

CALKINS, E. AND COHEN, A. S. 1959. Similarity of serum protein changes in primary and secondary amyloidosis. *J. Clin. Invest.* **38**: 993.

DE NAVASQUEZ, S., AND TREBLE, H. A. 1938. A case of primary generalized amyloid disease with involvement of nerves. *Brain* **61**: 116–128.

FALLS, H. F., JACKSON, C. E., CAREY, J. H., RUKAVINA, J. G. AND BLOCK, W. D. 1955. Ocular manifestations of hereditary primary systemic amyloidosis. *Arch. Ophth.* **54**: 660–664.

HELLER, H., SOHAR, E., AND SHERF, L. 1958. Familial Mediterranean fever. *Arch. Int. M.* 102: 50–71.

HORTA, J. DA S. 1956. Pathologische Anatomie der portugiesischen Paramyloidosenfälle mit besonderer Bevorzugung des peripheren Nervensystems. *Gaz. Méd. port.* 9: 678–699.

ISAAK, L. 1950. Localized amyloidosis cutis associated with psoriasis in siblings. *Arch. Derm. Syph.* 61: 859–862.

JACKSON, C. E., BLOCK, W. D., AND RATLIFF, W. C. 1960. Serum hexosamine content and urinary acid mucopolysaccharide excretion in hereditary primary amyloidosis. *J. Laborat. Clin. M.* 56: 544–546.

KANTARJIAN, A. D. AND DEJONG, R. N. 1953. Familial primary amyloidosis with nervous system involvement. *Neurology* 3: 399–409.

KAUFMAN, H. E. 1958. Primary familial amyloidosis. *Arch. Ophth.* **60**: 1036–1043.

KAUFMAN, H. E. AND THOMAS, L. B. 1959. Vitreous opacities diagnostic of familial primary amyloidosis. *New England J. M.* 261: 1267–1271.

MAXWELL, E. S. AND KIMBELL, I. 1936. Familial amyloidosis, with case reports. *Med. Bull. Veterans Admin.* 12: 365–369.

MAMOU, H. 1955. Maladie périodique amylogène. *Semaine hôp. Paris* **31**: 388–391.

NADEAU, L. A., MAGALINI, S. I. AND STEFANINI, M. 1956. Familial multiple myeloma. *Arch. Path.* 61: 101–106.

OSTERTAG, B. 1950. Familiare Amyloid-Erkrankung. *Ztschr. menschl. Vererb. u. Konstitutionslehre* 30: 105–115.

REIMANN, H. A., MOADIE, J., SEMERDJIAN, S. AND SAHYOUN, P. F. 1954. Periodic peritonitis-heredity and pathology; report of 72 cases. *J. Am. Med. Ass.* **154**: 1254–1259.

RIVA, G. 1958. Personal Communication.

RUKAVINA, J. G., BLOCK, W. D. AND CURTIS, A. C. 1956. Ultracentrifugal analyses of serum lipoproteins in familial primary systemic amyloidosis. *J. Laborat. Clin. M.* 47: 365–369.

RUKAVINA, J. G., BLOCK, W. D., JACKSON, C. E., FALLS, H. F., CAREY, J. H. AND CURTIS, A. C. 1956. Primary systemic amyloidosis: review and an experimental, genetic and clinical study of 29 cases with particular emphasis on the familial form. *Medicine* 35: 239–334.

SHULMAN, L. E. AND BARTTER, F. C. 1956. Familial primary amyloidosis. *Bull. Johns Hopkins Hosp.* 98: 238–239.

SMITHIES, O. 1955. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem. J.* 61: 629–641.

TUQAN, N. A. 1958. Periodic disease: a clinicopathologic study. *Ann. Int. M.* 49: 885–899.

VAN ALLEN, M. W. 1960. Personal Communication.

VIRCHOW, R. L. K. 1860. *Cellular pathology as based on physiological and pathological history*. New York: Robert DeWitt. 409–437.

VON SALLMAN, L., KAUFMAN, H. E., HAASE, G. R., BARTTER, F. C. AND THOMAS, L. B. 1960. Primary amyloidosis: Clinical staff conference at the National Institutes of Health. *Ann. Int. M.* 52: 668–681.

Secondary Male Hypogonadism and Congenital Ichthyosis: Association of Two Rare Genetic Diseases[1]

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INTRODUCTION

Hypogonadism in males refers both to deficient male hormone secretion by the testes as well as defective spermatogenesis. As seen clinically, no distinction can be made between primary or testicular and secondary or pituitary hypogonadism. The broad classification of hypogonadism has been reported by Albert *et al.* (1953) with the variety under consideration in our study being attributed to a selective pituitary lesion which is manifest prior to puberty. This concept is in accord with the terminology of "hypogonadotropic eunuchoidism" as used by Heller and Nelson (1948). Individuals with this condition are eunuchoid, have infantile secondary sexual characteristics, and have markedly low titers of pituitary gonadotropic hormones.

Diagnosis of male hypogonadism is usually made only in the extreme or frank cases. This is due to the unavailability to the clinician of gonadotropin assays and testicular biopsy interpretation plus a common neglect of this anatomic region on physical examination. Underdahl and Albert (1955) state that "... next to the male breasts, male gonads are the most commonly neglected structures during physical examination."

The genetic literature on male hypogonadism is limited with respect to investigations wherein proper distinction between the *primary* and *secondary* varieties have been made. Kallman *et al.* (1944) published an excellent review of the pertinent literature on the genetic aspects of "primary" hypogonadism extending back to 1902. However, in the light of recent advances in clinical endocrinology, it is believed that in most cases (surgical castration, diencephalic tumors, etc. excluded) gonadotropin assays must be used to make the final differentiation between the primary and secondary types. Of course, such techniques were not generally available at the time of the earlier investigations. The earliest genetic study that we have noted in the literature using gonadotropin assays is that of Hurxthal (1943) who reported pituitary (secondary) hypogonadism in 3 or 4 brothers. The rest of the family was reported as negative. Similar findings have since been reported for two male siblings by Ferriman

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(1950). Biben and Gordan (1953) reported five affected siblings in two families. One of the kindred had three males affected, while in the other, one brother and one sister were affected. Le Marquand (1957) studied a family in which three brothers and two sisters were affected. In each of the above reports using gonadotropin assays to confirm the diagnosis of *secondary* hypogonadism, an autosomal recessive mode of inheritance was advanced.

MATERIALS AND METHODS

The proband was extensively examined as an in-patient at the University of Texas Medical Branch Hospitals, Galveston, Texas and a diagnosis of secondary male hypogonadism and heriditary ichthyosis was made. Since his family history suggested the presence of other affected individuals, a "field trip" was organized to examine other members of the family. Our human genetics research team, composed of geneticists, internists, endocrinologists, and dermatologists, using clinic facilities in the patient's home town, made a thorough physical examination of twenty-six members of the family.

Diagnoses of secondary hypogonadism and congenital ichthyosis were made on the basis of careful physical examination as well as the following pertinent laboratory studies; assay for follicular stimulating hormone (FSH) by the method of Klinefelter; urinary 17-keto-steroids (method of Zimmerman); 17-hydroxy-corticosteroids (Porter-Silben method); serum PBI (modified Barker-Chaney method); and 24-hour thyroid I^{131} uptake studies (conventional method). In addition, testicular and skin biopsies were obtained where indicated and were interpreted by a member of the pathology department. In one case, a chromosome count was done by Dr. Yasushi Ohnuki working in the tissue culture laboratory of which Dr. C. M. Pomerat is director. Buccal smears were obtained on every patient examined and these were evaluated for sex chromatin by the method of Barr.

RESULTS

The proband (Fig. 1A and IV-14 Fig. 3) was a 19-year-old white male who appeared younger than his stated age, had a child-like face, was slightly obese, and had a moderate kyphosis. The principal findings on physical examination were those related to the hypogonadism and congenital ichthyosis. He had a typical eunuchoid habitus with a marked paucity of pubic and axillary hair. The penis was small (5 cm. in length and 4 cm. in circumference), and the scrotum showed a lack of pigmentation. The testes were both palpable but were less than ½ cm. in diameter. His voice was high pitched and child-like. The appearance of the skin over the extensor surfaces of the extremities was compatible with that of congenital ichthyosis and this clinical impression was later verified by skin biopsy. Testicular biopsy (Fig. 2) showed generalized atrophy, lack of spermatogenesis, almost total absence of Leydig cells and hyalinization of the conective tissue in some areas. Hormonal assay for urinary FSH showed a markedly low titer which confirmed the diagnosis of secondary hypogonadism. Other laboratory studies included urinary 17-keto-steroids, 17-hydroxy-corti-

FIG. 1. *A*, L. B. (IV-14) 19 years old. Note the absence of pubic and axillary hair, female fat distribution and eunuchoid habitus. Dark area on extensor surface of the lower extremities is due to congenital ichthyosis. *B*, G. H. R. (III-2) and *C*, T. R. (III-3) both show a similar eunuchoid habitus and manifest congenital ichthyosis.

costeroids, serum PBI, and 24-hour thyroid I^{131} uptake studies. These studies, with the exception of the I^{131} uptake, were found to be within normal limits which lends support to the diagnosis of selective pituitary gonadotropin failure. Values for these studies including those for the FSH titers are contained in table 1. In addition, buccal smears were obtained and were found to be negative for sex chromatin.

Positive diagnoses of secondary hypogonadism and congenital ichthyosis were likewise made on two maternal first cousins once removed (Fig. 1, B and C, and Fig. 3, III-2 and 3). Physical findings on these individuals were similar to those of the proband. Skin biopsies and testicular biopsies showed pathognomonic evidence for congenital ichthyosis and hypogonadism respectively. The laboratory studies (table 1) strongly supported the contention of a selective

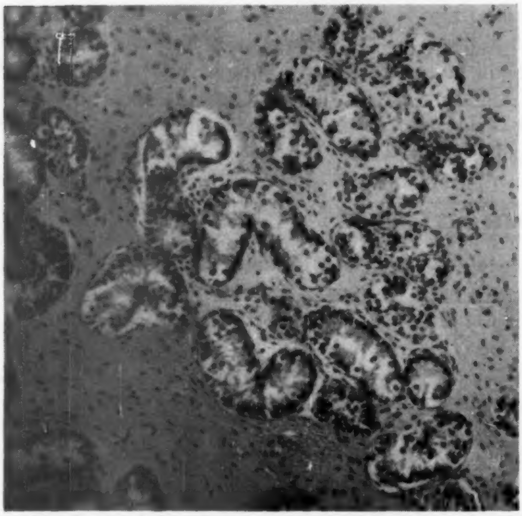

FIG. 2. Testicular biopsy. Evidence of marked generalized atrophy, cordlike seminiferous tubules, decreased Leydig cells and hyalinization in some areas.

TABLE 1. VALUES OF PERTINENT STUDIES FOR DIAGNOSIS OF SECONDARY MALE HYPOGONADISM

	Gonadotropins	17-KS mg/24 H	17-OH mg/24 H	PBI μg	I^{131}-Uptake 24-R%
Normal	Between 6 and 52 U	5–25	4–15	3.5–8	Between 12 and 45
L.R.B.	Less than 6.3 U	12.19–12.91	13.7–12.5	4.3	8
T.R.R.	Less than 6 U	22.7–23.4	6.4–9.17	8.8	12
G.H.R.	Greater than 6 U less than 16 U	13.8–12.7	—	4.9	—

pituitary gonatropin deficit. Buccal smears were negative for sex chromatin. A chromosome count was obtained on one of these individuals (G.H.R.) and this showed 46 chromosomes with an X Y chromosome constitution.

In addition, an exhausive family history revealed information which strongly suggests that two deceased maternal relatives (Fig. 3, II-3 and II-7) had shown features of hypogonadism and congenital ichthyosis which by description appear to be identical with that of the examined patients.

Physical examination of the remainder of the family (designated with +, Fig. 3) failed to show any definite suggestion of hypogonadism. Buccal smears from each individual showed no deviation from the phenotype. Of ancillary interest, but nevertheless of genetic importance, were findings of pectus excavatum, a cleft between the great and second toes, kyphosis and scoliosis. Examination of the pedigree (Fig. 3) shows the manner in which these traits are distributed.

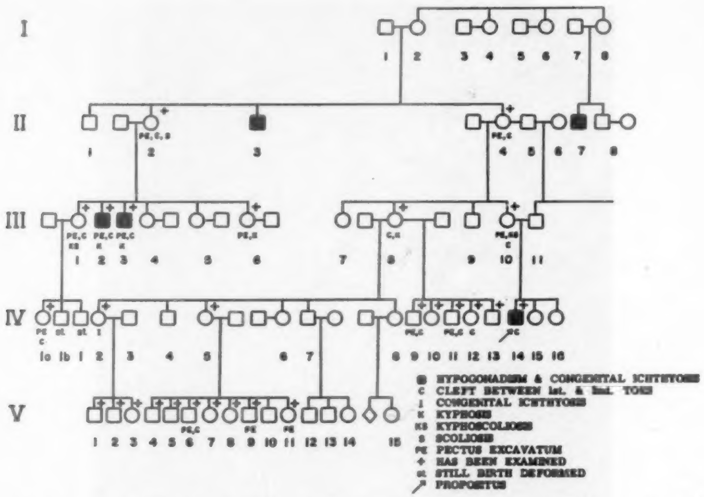

FIG. 3. Pedigree of family with secondary male hypogonadism and congenital ichthyosis.

DISCUSSION

A genetic analysis has been made of the several findings in this family. It was concluded that the mode of inheritance of the secondary hypogonadism is compatible with either a sex-linked recessive gene or an autosomal sex-limited dominant mode of inheritance. Examination of the pedigree shows that five females (I-2, I-8, II-2, II-4, III-10) appear to be carriers. Urinary gonadotropin assays obtained on two of these "carrier" females (II-2, III-10) showed completely normal titers.

Since the affected males in this family study are not fertile, an autosomal sex-limited dominant mode of inheritance cannot be excluded. Explanation for this alternative shall be advanced on a purely theoretical and speculative basis. It could involve an "anti-anterior pituitary" factor analogous to the "anti-testis" factor proposed originally by Witschi (1957) and later adopted by Lubs (1959), to give the etiological basis for hereditary male pseudohermaphrodism. Witschi suggests that this condition rests on a hereditary peculiarity of mothers in which the normal medullary function of the fetal testes is counteracted immediately following its embryonic differentiation, causing a genetically male fetus to develop into a male pseudohermaphrodite. Lubs (1959) reported a family in which there were five male pseudohermaphrodites in two generations with the females presumably acting as the carriers of a dominant gene but being themselves phenotypically normal. In light of this, it would be conceivable that instead of an "anti-testis" factor, an "anti-anterior pituitary" factor specifically inhibiting the gonadotropin secreting cells might exist, and thus secondarily cause testicular arrest. This would conceivably give rise to

secondary male hypogonadism and could readily mislead one into ascribing a sex-linked recessive mode of inheritance when in fact it might be an autosomal sex-limited dominant one. This hypothesis is advanced in hope of stimulating biochemical and histochemical research in this field.

A third possible genetic explanation for the inheritance of the syndrome in this family is that of a pleiotropic gene with variable expression which is more severe in males. This being the case, it might be reasoned that pectus excavatum, kyphosis, scoliosis, and toe clefts might be variable manifestations of the same mutant gene causing hypogonadism and congenital ichthyosis in males.

It is interesting to note that congenital ichthyosis has been previously described as following a sex-linked recessive mode of inheritance (Gates, 1948; Lewis, 1959). However, in the present family congenital ichthyosis appears in association with secondary hypogonadism in the males and could be behaving as a linkage phenomenon. A review of the medical and gentic literature (Lynch *et al.*, 1960) indicates this to be the first reported example of these conditions being transmitted concomitantly through more than one generation. If this combination is actually a linkage phenomenon with both genes on the X chromosome, then profound diagnostic and ultimate therapeutic gain could be achieved. The reason for the importance of this point is that the ichthyosis is frequently manifest at the usual sites (extensor surfaces) within the first two years of life. Hence, the "marker gene" of ichthyosis would permit the clinician to have advantage of early detection of hypogonadism, as suggested by Kupperman and Epstein (1958), permitting hormonal replacement therapy to be started earlier. There is, however, an exceedingly rare condition known as the syndrome of Rüd with only seven reported cases in the world literature (MacGillivray, 1954) in which congenital ichthyosis and secondary hypogonadism occur as concomitant findings. However, there are other features of this syndrome, including severe mental deficiency and epilepsy which clearly differentiate this condition from that found in the present investigation. The possibilty that the combination of secondary hypogonadism and congenital ichthyosis, as manifested in this family, might represent a distinct and new syndrome (as opposed to a linkage phenomenon) has been considered. However, we feel that there is not sufficient evidence at this time to establish this possibility.

Several additional findings of genetic significance were observed in the present study, most prominent of which was pectus excavatum. This condition consists of a depression of the inferior portion of the sternum which may be clinically manifest at birth but which frequently arises later (Lester, 1957, 1958). The degree of expression of this condition varies considerably and mild asymptomatic forms are most common. An autosomal dominant mode of inheritance has been previously suggested for this condition (Gates, 1948). This is consistent with the transmission in this family in which the condition appeared in four generations (II-2, III-1, IV-9, and V-6 among others). Another finding was the presence of a large cleft between the first and second toes bilaterally. This was present in 14 individuals in four generations and also appears to be transmitted as an autosomal dominant gene with complete expression. Further evidence of genetic

skeletal abnormalities was manifest in the form of kyphosis, scoliosis, and kyphoscoliosis found in 8 members of the family through three generations. However, there is not sufficient data to evaluate the genetic significance of this finding adequately, although it appears to be behaving as an autosomal dominant. Those for whom these diagnoses were recorded exhibited the conditions prominently. Recognizing the obvious limitations of clinical judgement, as opposed to X-ray evaluation in detecting minimal kyphosis and scoliosis, we believe that X-ray studies on all members of the family would supply sufficient information for a more refined genetic interpretation of these interesting findings.

SUMMARY

A family with secondary or pituitary male hypogonadism and congenital ichthyosis was studied. These diagnoses were confirmed on three individuals by urinary gonadotropin assays, testicular biopsies, skin biopsies, buccal smears, urinary 17-keto and hydroxy-steroids, serum PBI's, and studies of I^{131} uptake. Two deceased maternal relatives are believed on the basis of history, to have had both hypogonadism and congenital ichthyosis. There were additional observations of pectus excavatum, cleft between the first and second toes, kyphosis, scoliosis, and kyphoscoliosis. A sex-linked recessive mode of inheritance is advanced for the secondary male hypogonadism and the congenital ichthyosis with the view that these characters may be linked on the X chromosome. Other possible modes of inheritance for this combination have been discussed.

ACKNOWLEDGEMENTS

We are indebted to Dr. Raymond F. Blount of the Department of Anatomy at the Medical Branch for his most careful and critical reading of the manuscript.

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REFERENCES

Albert, A., Underdahl, L. O., Greene, L. F. and Lorenz, N. 1953. Male hypogonadism. II. Classification. *Proc. Mayo Clin.* 28: 557–567.

Biben, R. L. and Gordan, G. S. 1955. Familial hypogonadotropic eunuchoidism. *J. Clin. Endocr. Metab.* 15: 931–942.

Ferriman, D. G. 1950. Gynaecomastia and testicular aplasia. *Brit. M. J.* 1: 162–163.

Gates, R. R. 1948. *Human Genetics.* Vol. I. New York: The Macmillan Company.

Heller, C. G. and Nelson, W. O. 1948. Classification of male hypogonadism and a discussion of the pathologic physiology, diagnosis, and treatment. *J. Clin. Endocr.* 8: 345–366.

Hurxthal, L. M. 1943. Sublingual use of testosterone in seven cases of hypogonadism: Report of three congenital eunuchoids occurring in one family. *J. Clin. Endocr.* 3: 551–556.

Kallman, F. J., Schonfeld, W. A. and Barrera, S. E. 1944. The genetic aspects of primary eunuchoidism. *Am. J. Ment. Defic.* 48: 203–236.

Kupperman, H. S. and Epstein, J. A. 1958. Hormonal therapy versus watchful waiting in hypogonadism: The male. *J. Am. Geriat. Soc.* 6: 87–98.

LeMarquand, H. S. 1954. Congenital hypogonadotrophic hypogonadism in five members of a family, three brothers and two sisters. *Proc. R. Soc. Lond.* **47**: **442–448.**

Lester, C. W. 1957. The etiology and pathogenesis of funnel chest, pigeon breast, and related deformities of the anterior chest wall. *J. Thorac. Surg.* **34**: **1–10.**

Lester, C. W. 1958. The relations of pectus excavatum to pectus carinatum: Classification of anterior chest wall deformities and the effect on treatment. *J. Pediat., St. Louis.* **52**: **82–86.**

Lewis, G. M. 1959. *Practical Dermatology*. 2nd. Edition. Philadelphia: W. B. Saunders Co.

Lubs, H. A. Jr., Vilar, O. and Bergenstal, D. M. 1959. Familial male pseudohermaphrodism with labial testes and partial feminization: Endocrine studies and genetic aspects. *J. Clin. Endocr. Metab.* 19: 1110–1120.

Lynch, H. T., McNutt, C. W., Johnson, J. E., Ozer, F. L. and Jampolsky, N. A. 1960. Hereditary secondary male hypogonadism: A family study. Paper presented to meeting of Am. Soc. Human Genetics, Memphis, Tennessee, April, 1960.

MacGillivray, R. C. 1954. The syndrome of Rüd. *Am. J. Ment. Defic.* **59**: **62–72.**

Underdahl, L. O. and Albert, A. 1955. Male hypogonadism. *Postgrad. M.* 17: 251–258.

Witschi, W., Nelson, W. O. and Segal, S. J. 1957. Genetic, developmental and hormonal aspects of gonadal dysgenesis and sex inversion in man. *J. Clin. Endocr. Metab.* **17**: 737–753.

BOOK REVIEWS

Inheritance of Glioma: The Genetic Aspects of Cerebral Glioma and Its Relation to Status Dysraphicus

By H. J. VAN DER WIEL. New York: Van Nostrand Co. Inc., 1960, 275 pp. $12.50.

THIS BOOK, in English, is made up of 275 pages, 19 of which are bibliography; 26, review of genetics of neoplasms in general; 128, detailed clinical findings in the relatives of 100 probands with cerebral glioma. There were also 100 probands without glioma whose names were chosen "at random" whose relatives served as controls. Many of the associated conditions supposed to be a part of the syndrome differed widely in their degree of expressivity. The decision as to whether they should be included in the case of specifically affected relatives or excluded would be purely subjective. It was desirable, therefore, that one investigator examine the relatives of affected and control probands and at the same time in the investigation. This proved impracticable, and the relatives of 50 of the control probands were examined by a colleague of the author.

The relatives comprised grandparents, parents, aunts and uncles, sibs, cousins, children and grandchildren of the probands. Living relatives were given a most detailed examination, and the causes of death of the decreased were obtained, where possible, from the attending physician. As will be seen from the list of stigmata which are thought by the author to be possibly a part of the status dysraphicus syndrome, (an abnormal fusion of the embryonic dorsal aspect of the central nervous system with attendant sequelae) the presence of many of these associated stigmata in the deceased relatives would be unrecorded. Also, many of them do not appear until adult life, hence not likely to be found, especially in grandchildren who would be young.

The list of so-called primary and accessory stigmata is astonishingly long, and it would not be surprising if, on the basis of chance alone, some of them were found to have a significant correlation with glioma without there being any real association. Also some appear (at least to the reviewer) to have little or no relation to possible disturbance of fusion of the CNS. As primary stigmata he lists spina bifida, kyphoscoliosis and other disturbances of the vertebral column, funnel chest, hollow feet or unduly high arches, club foot, cyanosis of the hands and feet, absence or disturbance of reflexes, trophic ulcer, enuresis, Horner's syndrome associated with heterochromia iridis. The accesory stigmata are clinodactyly, campodactyly, webbed fingers and toes, winged scapulae, inequality in the size of the breasts, supernumerary nipples, span of the arms exceeding the height by a ratio of more than 108, harelip, cleft palate, unduly high palate, lumbosacral hypertrichosis, anomalies of jaws and teeth, café-au-lait spots (CAL) and what the author terms "animated skin", i.e., skin with a "strikingly numerous" amount of small naevi, pedunculated small fibromata, angiomas, etc. The author also considered the mongoioid palm line (FFL) hallux valgus, nystagmus, hypermobility of the joints, flared out ears, club thumbs among the conditions to be investigated. At once the subjective element in diagnosis becomes apparent. When is a palate or the arch of the foot "unduly" high? When is the tip of the little finger bent at an angle sufficiently great to cause that relative to be included among the stigmatized? The author warns against including minor or lesser cases of stigmata among those to be accepted, lest one "prove too much". (The

reviewer feels that more reliance could be placed upon the various estimates of significance of frequency with which these stigmata were found in the relatives of the glioma probands as contrasted with the relatives of the controls, had an investigator examined the two groups of relatives with no knowledge of the gliomatous state of the proband.)

Thirty-seven tables give a detailed analysis of the frequency of these various stigmata in the relatives of the two groups. Most of the stigmata were more frequent in the relatives of the affected probands than in the relatives of the controls. They were also more frequent in the close relatives of the affected probands than among their cousins. Most of the conditions listed above were included in the status dysraphicus category, and it was concluded that this condition predisposes to the development of cerebral or cerebellar glioma.

Although he starts his chapter on his own research with the statement that within two months he found three patients who were suspected of having brain tumor with a family history of other similarly affected persons, his Table 9 lists only one family of his own group where a grandfather and granddaughter had glioma. This made only one per cent of his families with more than one with actual glioma.

A division of people into three groups was made, (1) the truly non-dysraphic, (2) those with one primary and not more than one accessory stigmata, or without any primary but with no more than three accessory stigmata, and (3) those with more stigmata than in group 2. When both parents belonged to group 1 there were no dysraphic children. When both parents belonged to group 3, there were no truly non-dysraphic children. On the basis of these findings he concludes that he has demonstrated the inheritance of status dysraphicus and that the figures suggest dominant transmission.

MADGE T. MACKLIN

Human Heredity

By ASHLEY MONTAGU. Cleveland: World Publishing Company, 1959, 397 pages, $5.00.

SINCE this book was written for the general reader, it is not surprising that it is most similar to Amram Scheinfeld's "The New You and Heredity". The author presents the basic principles of genetics in the first three chapters and in the next four discusses various aspects of hereditary and environmental interactions, dispelling at the same time some of the common misconceptions regarding heredity. The next five chapters discuss twins, crime, constitution, sex, and race. Part two of the book entitled "The Family Album" discusses inherited characteristics of the human body and a couple of special topics such as radiation. The book includes five appendices, a table of inherited disorders of man, a page devoted to consanguineous unions, a third listing counseling centers in the United States, a glossary, and, finally, one on Genetics in the U.S.S.R.

One express purpose of the author is to correct the impression he believes biologists create in writing about heredity, "... that the genes and the chromosomes are all." Though this reader would take issue with his premise, it seems to me that the author has not achieved his avowed purpose. In the first few chapters he has emphasized the role that the environment plays in the development of human traits, though in some cases he might have chosen better examples. However, the remainder of the book seems to have been written in the same manner as that which he finds reprehensible when

used by biologists. Thus, on page 237 we read, "The character of the eyelashes, their thickness, length and curvature are all inherited traits . ."

The book is also marred by a number of dogmatic statements presented as refutations of prejudicial conceptions. For instance, on page 65 the author states, "The blood of all human beings is in every respect the same, except for variations in the frequencies with which the blood group factors, the Rh factors and the sickle-cell factors occur." Aside from other differences already known, who can say how many additional ones remain to be discovered? In Chapter 5 he erroneously creates an impression of a clear and present danger when discussing the influence of the maternal environment on the developing fetus by mentioning radiation as one example and in the next sentence, "loud sounds". He suggests that both are capable of "... seriously affecting the development of the organism."

As an example of the device to which the author often resorts in minimizing rather than evaluating the role of genetic factors, we read the following on tuberculosis, "The probability of a genetic factor connected with tuberculosis is therefore high, and it would appear likely that many genes are involved. However, it is clear from these same twin studies that in the discordant cases where there is a difference in the health of the twins it is invariably the *weaker twin* (italics supplied mine) who is affected. So the resistance is not entirely genetically determined."

A further example of polemics rather than scientific argument is exemplified by the following from his chapter on crime: "How does it happen that drunkenness is a condition that occurs so rarely among Jews? Is it because these groups have few or no genes disposing toward such behavior? The answer is positively in the negative." Unfortunately, throughout the book the author uses strong statements most frequently where scientific information is either scanty or wholly lacking.

In summary, the book could have been half its size and double its quality if it had been less redundant and had omitted those areas in which little definitive knowledge is presently available. Although the book includes some more recent findings than Scheinfeld's, I would recommend the latter for the general reader in preference to Montagu's book.

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Heredity and Human Nature

By David C. Rife. New York: Vantage Press, 1959, 265 pp., $4.50.

This book was written to demonstrate that the appraisal of genetic variability can make an important contribution to the understanding of human nature. The author directs his attention toward those who are "inclined to attribute all differences in abilities and achievements to inequalities in environment," and insists in a variety of ways that "there is no good reason for being alarmed about and reluctant to discuss normal innate human variations. They should, instead, provide a source of confidence in the future of mankind, and an appreciation of them should make us more charitable in judgments of our fellow men."

This book turns out to be a presentation of basic genetics at a fairly elementary

level with interludes and applications in the style of a personal essay. After a review of biological differences between male and female and of simple patterns of inheritance, there is a discussion of interactions between genes and environment, followed by a presentation of population genetics and the topics of inbreeding and hybrid vigor. The last third of the book considers "melting pots" and the importance of heredity in behavior and human nature. The author appropriately includes his own research interests—the study of mixed Negro-white populations, evidence for genetic factors in handedness, the importance of dermatoglyphics, and studies of twins.

A wide range of genetic detail is presented in a style adapted for a lay audience. The choice of details is acceptable for the purposes of the book, but some geneticists may feel that the style of writing is not careful enough. The use of phrases such as "everyone knows," "invariably," or "undoubtedly" arouses questions as to the evidence for the statements. Some shortcuts in reasoning are employed. The fact that human beings have twenty-three pairs of chromosomes, for example, is not by itself proof that all belong to the same species. In the understandable effort to simplify the language, some necessary exceptions or explanations appear to have been omitted in statements such as the following: "Sex depends upon a block of genes which occur doubly in females and singly in males" (p. 25). "Unlike a recessive gene, a dominant gene does not skip generations" (p. 46).

A similar need for precision is sensed when topics in education, sociology, psychology, and political science are considered. In his effort to show the relevance of genetics, the author discusses the concept, definition, and ideals of democracy in a manner that probably will seem naive to at least some social scientists, and thereby weakens his argument. The attempt to settle an issue by getting more evidence and weighing it more carefully, for example, cannot be considered a part of the "democratic method." Furthermore, to ascribe the prevalence of an "environmentalist" point of view to the success of communist propaganda is misleading and inadequate. In any effort to convince persons in another discipline (and often with a different orientation) it is important to use terms and facts carefully.

Several errors which might prove confusing to readers should be mentioned. On page 25 the phrase should be "sex ratios within *families*," not *females*. In the second table on page 54 the blood type of the father on the first line should be "A or AB" and on the second line should be "M or MN." On page 159 the line should read: "there is *one* (instead of *no*) chance in four." In addition, some pedigrees are poorly drawn or too small, and some tables and figures are inadequately labeled.

The reservations which geneticists or social scientists may have must not be allowed to detract from the very real value the book can be expected to have for laymen. The fact that biological components are involved in human behavior needs repeated emphasis, and the citizens in a democracy should be informed of the need for further research in this area. There are many practical implications in the observation that most human variations (especially those which are quantitative in nature) are the products of both heredity and environment. Those who may think of the United States as a melting pot must recognize that while cultural differences diminish, the total genetic variability will tend to remain constant. The task of applying our knowledge of genetics to the understanding of human nature is an important one, and the author of this book has much to contribute.

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BOOKS RECEIVED

Reptiles: Life History, Evolution, and Structure

By ANGUS BELLAIRS. Harper Torchbooks/The Science Library. New York: Harper and Brothers, 1960, 192 pp., $1.35.

THIS is a reprint of a book that was first published in 1957 by Hutchinson and Company. Its purpose is to provide a synthesis of the different sorts of knowledge available on reptiles.

Animal Species and Their Evolution

By A. J. CAIN. Harper Torchbooks/The Science Library. New York: Harper and Brothers, 1960, 190 pp., $1.35.

THIS is a reprint of a book that was first published in 1954 by Hutchinson and Company. It is intended to be a survey of the nature of species, their origin, and their evolutionary importance. It is assumed that the reader does not have any previous knowledge of the subject.

Problems of Life

By LUDWIG VON BERTALANFFY. Harper Torchbooks/The Science Library. New York: Harper and Brothers, 1960, 216 pp., $1.35.

THIS is a reprint of a book that was first published in 1952 by C. A. Watson Company. The book is an exposition and development of the author's viewpoint known as the *organismic conception*. It presents a survey of basic biological problems and laws within the framework of the organismic conception. It proceeds from this to arrive at the general principles of the modern conception of the world, and the claim for a "General System Theory".

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